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Date of mailing (day/month/year) 14 January 2000 (14.01.00)	
International application No. PCT/US99/10793	**Applicant's or agent's file reference** 220002057756
International filing date (day/month/year) 14 May 1999 (14.05.99)	**Priority date** (day/month/year) 14 May 1998 (14.05.98)
Applicant GATANAGA, Tetsuya et al	

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Form PCT/IB/331 (July 1992)

3052077

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/00 | A2

(11) International Publication Number: **WO 99/58559**

(43) International Publication Date: 18 November 1999 (18.11.99)

(21) International Application Number: PCT/US99/10793

(22) International Filing Date: 14 May 1999 (14.05.99)

(30) Priority Data:
09/081,385 14 May 1998 (14.05.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: FACTORS AFFECTING TUMOR NECROSIS FACTOR RECEPTOR RELEASING ENZYME ACTIVITY

(57) Abstract

The biological effects of the cytokine TNF are mediated by binding to receptors on the surface of cells. This disclosure describes new proteins and polynucleotides that promote enzymatic cleavage and release of TNF receptors. Also provided are method for identifying additional compounds that influence TNF receptor shedding. As the active ingredient in a pharmaceutical composition, the products of this invention increase or decrease TNF signal transduction, thereby alleviating the pathology of disease.

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FACTORS AFFECTING TUMOR NECROSIS FACTOR RECEPTOR RELEASING ENZYME ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of U.S. application 09/081,385, filed May 14, 1998, pending. For purposes of prosecution in the U.S., the priority application is hereby incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This invention relates generally to the field of signal transduction between cells, via cytokines and their receptors. More specifically, it relates to enzymatic activity that cleaves and releases the receptor for TNF found on the cell surface, and the consequent biological effects. Certain embodiments of this invention are compositions that affect such enzymatic activity, and may be included in medicaments for disease treatment.

BACKGROUND OF THE INVENTION

Cytokines play a central role in the communication between cells. Secretion of a cytokine from one cell in response to a stimulus can trigger an adjacent cell to undergo an appropriate biological response — such as stimulation, differentiation, or apoptosis. It is hypothesized that important biological events can be influenced not only by affecting cytokine release from the first cell, but also by binding to receptors on the second cell, which mediates the subsequent response. The invention described in this patent application provides new compounds for affecting signal transduction from tumor necrosis factor.

The cytokine known as tumor necrosis factor (TNF or TNF-α) is structurally related to lymphotoxin (LT or TNF-β). They have about 40 percent amino acid sequence homology (Old, *Nature* 330:602-603, 1987). These cytokines are released by macrophages, monocytes and natural killer cells and

play a role in inflammatory and immunological events. The two cytokines cause a broad spectrum of effects both in vitro and in vivo, including: (i) vascular thrombosis and tumor necrosis; (ii) inflammation; (iii) activation of macrophages and neutrophils; (iv) leukocytosis; (v) apoptosis; and (vi) shock. TNF has been associated with a variety of disease states including various forms of cancer, arthritis, psoriasis, endotoxic shock, sepsis, autoimmune diseases, infections, obesity, and cachexia. TNF appears to play a role in the three factors contributing to body weight control: intake, expenditure, and storage of energy (Rothwell, *Int. J. Obesity* 17:S98-S101, 1993). In septicemia, increased endotoxin concentrations appear to raise TNF levels (Beutler et al. *Science* 229:869-871, 1985).

Attempts have been made to alter the course of a disease by treating the patient with TNF inhibitors, with varying degrees of success. For example, the TNF inhibitor dexanabinol provided protection against TNF mediated effects following traumatic brain injury (Shohami et al. *J. Neuroimmun.* 72:169-77, 1997). Some improvement in Crohn's disease was afforded by treatment with anti-TNF antibodies (Neurath et al., *Eur. J. Immun.* 27:1743-50, 1997).

Human TNF and LT mediate their biological activities by binding specifically to two distinct glycoprotein plasma membrane receptors (55 kDa and 75 kDa in size, known as p55 and p75 TNF-R, respectively). The two receptors share 28 percent amino acid sequence homology in their extracellular domains, which are composed of four repeating cysteine-rich regions (Tartaglia and Goeddel, *Immunol. Today* 13:151-153, 1992). However, the receptors lack significant sequence homology in their intracellular domains, and mediate different intracellular responses to receptor activation. In accordance with the different activities of TNF and LT, most human cells express low levels of both TNF receptors: about 2,000 to 10,000 receptors per cell (Brockhaus et al., *Proc. Natl. Acad. Sci.* USA *87:3127-3131,* 1990).

Expression of TNF receptors on both lymphoid and non-lymphoid cells can be influenced experimentally by many different agents, such as bacterial lipopolysaccharide (LPS), phorbol myristate acetate (PMA; a protein kinase C

activator), interleukin-1 (IL-1), interferon-gamma (IFN-γ) and IL-2 (Gatanaga et al. *Cell Immunol.* 138:1-10, 1991; Yui et al. *Placenta* 15:819-835, 1994). It has been shown that complexes of human TNF bound to its receptor are internalized from the cell membrane, and then the receptor is either degraded or recycled (Armitage, *Curr. Opin. Immunol.* 6:407-413, 1994). It has been proposed that TNF receptor activity can be modulated using peptides that bind intracellularly to the receptor, or which bind to the ligand binding site, or that affect receptor shedding. See for example patent publications WO 95/31544, WO 95/33051, WO 96/01642, and EP 568 925.

TNF binding proteins (TNF-BP) have been identified at elevated levels in the serum and urine of febrile patients, patients with renal failure, and cancer patients, and even certain healthy individuals. Human brain and ovarian tumors produced high serum levels of TNF-BP These molecules have been purified, characterized, and cloned (Gatanaga et al., *Lymphokine Res.* 9:225-229, 1990a; Gatanaga et al., *Proc. Natl. Acad. Sci USA* 87:8781-8784, 1990b). Human TNF-BP consists of 30 kDa and 40 kDa proteins which are identical to the N-terminal extracellular domains of p55 and p75 TNF receptors, respectively (US Patent No. 5,395,760; EP 418,014). Such proteins have been suggested for use in treating endotoxic shock. Mohler et al. *J. Immunol.* 151:1548–1561, 1993

There are several mechanisms possible for the production of secreted proteins resembling membrane bound receptors. One involves translation from alternatively spliced mRNAs lacking transmembrane and cytoplasmic regions. Another involves proteolytic cleavage of the intact membrane receptors, followed by shedding of the cleaved receptor from the cell. The soluble form of p55 and p75 TNF-R do not appear to be generated from mRNA splicing, since only full length receptor mRNA has been detected in human cells *in vitro* (Gatanaga et al., 1991). Carboxyl-terminal sequencing and mutation studies on human p55 TNF-R indicates that a cleavage site may exist between residues Asn 172 and Val 173 (Gullberg et al. *Eur. J. Cell. Biol.* 58:307-312, 1992).

There are reports that a specific metalloprotease inhibitor, TNF-α protease inhibitor (TAPI) blocks the shedding of soluble p75 and p55 TNF-R (Crowe et al.

J. Exp. Med. 181:1205-1210, 1995; Mullberg et al. *J. Immunol.* 155:5198-5205, 1995). The processing of pro-TNF on the cell membrane to release the TNF ligand appears to be dependent on a matrix metalloprotease like enzyme (Gearing et al. *Nature* 370:555-557, 1994). This is a family of structurally related matrix-degrading enzymes that play a major role in tissue remodeling and repair associated with development and inflammation (Birkedal-Hansen et al. *Crit. Rev. Oral Biol. Med.* 4:197–250, 1993). The enzymes have Zn^{2+} in their catalytic domains, and Ca^{2+} stabilizes their tertiary structure significantly.

In European patent application EP 657536A1, Wallach et al. suggest that it would be possible to obtain an enzyme that cleaves the 55,000 kDa TNF receptor by finding a mutated form of the receptor that is not cleaved by the enzyme, but still binds to it. The only proposed source for the enzyme is a detergent extract of membranes for cells that appear to have the protease activity. If it were possible to obtain an enzyme according to this scheme, then the enzyme would presumably comprise a membrane spanning region. The patent application does not describe any protease that was actually obtained.

In a previous patent application in the present series (International Patent Publication WO 9820140), methods are described for obtaining an isolated enzyme that cleaves both the p55 and p75 TNF-R from cell surfaces. A convenient source is the culture medium of cells that have been stimulated with phorbol myristate acetate (PMA). The enzyme activity was given the name TRRE (TNF receptor releasing enzyme). In other studies, TRRE was released immediately upon PMA stimulation, indicating that it is presynthesized in an inactive form to be rapidly converted to the active form upon stimulation. Evidence for direct cleavage of TNF-R is that the shedding begins very quickly (~5 min) with maximal shedding within 30 min. TRRE is specific for the TNF-R, and does not cleave IL-1 receptors, CD30, ICAM-1 or CD11b. TRRE activity is enhanced by adding Ca^{++} or Zn^{++}, and inhibited by EDTA and phenantroline.

Given the involvement of TNF in a variety of pathological conditions, it is desirable to obtain a variety of factors that would allow receptor shedding to be

modulated, thereby controlling the signal transduction from TNF at a disease site.

SUMMARY OF THE INVENTION

This disclosure provides new compounds that promote enzymatic cleavage and release of TNF receptors from the cell surface. Nine new DNA clones have been selected after repeat screening in an assay that tests the ability to enhance receptor release. The polynucleotide sequences of this invention and the proteins encoded by them have potential as diagnostic aids, and therapeutic compounds that can be used to adjust TNF signal transduction in a beneficial way.

One embodiment of the invention is an isolated polynucleotide comprising a nucleotide sequence with the following properties: a) the sequence is expressed at the mRNA level in Jurkat T cells; b) when COS-1 cells expressing TNF-receptor are genetically transformed to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor. If a polynucleotide sequence is expressed in Jurkat cells, then it can be found in the Jurkat cell expression library deposited with the ATCC (Accession No. TIB-152). It is recognized that the polynucleotide can be obtained from other cell lines, or produced by recombinant techniques.

Included are polynucleotides in which the nucleotide sequence is contained in any of SEQ. ID NOS:1-10. Also embodied are polynucleotides comprising at least 30 and preferably more consecutive nucleotides in said nucleotide sequence, or at least 50 consecutive nucleotides that are homologous to said sequence at a significant level, preferably at the 90% level or more. Also included antisense and ribozyme polynucleotides that inhibit the expression of a TRRE modulator.

Another embodiment of the invention is isolated polypeptides comprising an amino acid sequence encoded by a polynucleotide of this invention. Non-limiting examples are sequences shown in SEQ. ID NOS: 147-158. Fragments

and fusion proteins are included in this invention, and preferably comprise at least 10 consecutive residues encoded by a polynucleotide of this invention, or at least 15 consecutive amino acids that are homologous at a significant level, preferably at least 80%. Preferred polypeptides promote cleavage and release of TNF receptors from the cell surface, especially COS-1 cells genetically transformed to express TNF receptor. The polypeptides may or may not have a membrane spanning domain, and may optionally be produced by a process that involves secretion from a cell. Included are species homologs with the desired activity, and artificial mutants with additional beneficial properties.

Another embodiment of this invention is an antibody specific for a polypeptide of this invention. Preferred are antibodies that bind a TRRE modulator protein, but not other substances found in human tissue samples in comparable amounts.

Another embodiment of the invention is an assay method of determining altered TRRE activity in a cell or tissue sample, using a polynucleotide or antibody of this invention to detect the presence or absence of the corresponding TRRE modulator. The assay method can optionally be used for the diagnosis or evaluation of a clinical condition relating to abnormal TNF levels or TNF signal transduction.

Another embodiment of the invention is a method for increasing or decreasing signal transduction from a cytokine into a cell (including but not limited to TNF), comprising contacting the cell with a polynucleotide, polypeptide, or antibody of this invention.

A further embodiment of the invention is a method for screening polynucleotides for an ability to modulate TRRE activity. The method involves providing cells that express both TRRE and the TNF-receptor; genetically altering the cells with the polynucleotides to be screened; cloning the cells; and identifying clones with the desired activity.

Yet another embodiment of the invention is a method for screening substances for an ability to affect TRRE activity. This typically involves incubating cells expressing TNF receptor with a TRRE modulator of this

invention in the presence or absence of the test substance; and measuring the effect on shedding of the TNF receptor .

The products of this invention can be used in the preparation of a medicament for treatment of the human or animal body. The medicament contains a clinically effective amount for treatment of a disease such as heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, sepsis, and cancer. These compositions can be used for administration to a subject suspected of having or being at risk for the disease, optionally in combination with other forms of treatment appropriate for their condition.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of plasmid pCDTR2. This plasmid expresses p75 TNF-R, the ~75 kDa form of the TNF receptor. PCMV stands for cytomegalovirus; BGHpA stands for bovine growth hormone polyadenylation signal.

Figure 2 is a line depicting the levels of p75 TNF-R detected on COS-1 cells genetically altered to express the receptor. Results from the transformed cells, designated C75R (●, upward swooping line) is compared with that from the parental COS-1 cells (■, baseline). The receptor number was calculated by Scatchard analysis (inset).

Figure 3 is a survival graph, showing that TRRE decreases mortality in mice challenged with lipopolysaccharide (LPS) to induce septic peritonitis. (◆) LPS alone; (■) LPS plus control buffer; (●) LPS plus TRRE (2,000 U); (▲) LPS plus TRRE (4,000 U).

Figure 4 is a half-tone reproduction of a bar graph, showing the effect of 9 new clones on TRRE activity on C75R cells (COS-1 cells transfected to express the TNF-receptor. Each of the 9 clones increases TRRE activity by over 2-fold.

Figure 5 is a survival graph, showing the ability of 4 new expressed to save mice challenged with LPS. (◆) saline; (■) BSA; (△) Mey-3 (100 μg); (X) Mey-3 (10 μg); (*) Mey-5 (10 μg); (●) Mey-8 (10 μg).

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that certain cells involved in the TNF transduction pathway express enzymatic activity that causes TNF receptors to be shed from the cell surface. Enzymatic activity for cleaving and releasing TNF receptors has been given the designation TRRE. Phorbol myristate acetate induces release of TRRE from cells into the culture medium. An exemplary TRRE protein had been purified from the supernatant of TNF-1 cells (Example 2). The protease bears certain hallmarks of the metalloprotease family, and is released rapidly from the cell upon activation.

In order to elucidate the nature of this protein, functional cloning was performed. Jurkat cells were selected as being a good source of TRRE. The cDNA from a Jurkat library was expressed, and cell supernatant was tested for an ability to release TNF receptors from cell surfaces. Cloning and testing of the expression product was conducted through several cycles, and nine clones were obtained that more than doubled TRRE activity in the assay (Figure 4). At the DNA level, all 9 clones had different sequences.

Protein expression products from the clones have been tested in a lipopolysaccharide animal model for sepsis. Protein from three different clones successfully rescued animals from a lethal dose of LPS (Figure 5). This points to an important role for these molecules in the management of pathological conditions mediated by TNF.

The number of new TRRE promoting clones obtained from the expression library was surprising. The substrate specificity of the TRRE isolated in Example 2 distinguishes the 75 kDa and 55 kDa TNF receptors from other cytokine receptors and cell surface proteins. There was little reason beforehand to suspect that cells might have nine different proteases for the TNF receptor. It is possible that one of the clones encodes the TRRE isolated in Example 2, or a related protein. It is possible that some of the other clones have proteolytic activity to cleave TNF receptors at the same site, or at another site that causes release of the soluble form from the cell. It is a hypothesis of this disclosure that some of the clones may not have proteolytic activity themselves, but play a role in promoting TRRE activity in a secondary fashion.

This possibility is consistent with the observations made, because there is an endogenous level of TRRE activity in the cells used in the assay. The cleavage assay involves monitoring TNF receptor release from C75 cells, which are COS-1 cells genetically altered to express p75 TNF-R. The standard assay is conducted by contacting the transformed cells with a fluid believed to contain TRRE. The level of endogenous TRRE activity is evident from the rate of spontaneous release of the receptor even when no exogenous TRRE is added (about 200 units). Accordingly, accessory proteins that promote TRRE activity would increase the activity measured in the assay. Many mechanisms of promotion are possible, including proteins that activate a zymogen form of TRRE, proteins that free TRRE from other cell surface components, or proteins that stimulate secretion of TRRE from inside the cell. It is not necessary to understand the mechanism in order to use the products of this invention in most of the embodiments described.

It is anticipated that several of the clones will have activity not just for promoting TNF receptor cleavage, but also having an effect on other surface proteins. To the extent that cleavage sequences or accessory proteins are shared between different receptors, certain clones would promote phenotypic change (such as receptor release) for the family of related substrates.

This disclosure provides polypeptides that promote TRRE activity, polynucleotides that encode such polypeptides, and antibodies that bind such peptides. The binding of TNF to its receptor mediates a number of biological effects. Cleavage of the TNF-receptor by TRRE diminishes signal transduction by TRRE. Potentiators of TRRE activity have the same effect. Thus, the products of this invention can be used to modulate signal transduction by cytokines, which is of considerable importance in the management of disease conditions that are affected by cytokine action. The products of this invention can also be used in diagnostic methods, to determine when signal transduction is being inappropriately affected by abnormal TRRE activity. The assay systems described in this disclosure provide a method for screening additional compounds that can influence TRRE activity, and thus the signal transduction from TNF.

Based on the summary of the invention, and guided by the illustrations in the example section, one skilled in the art will readily know what techniques to employ in the practice of the invention. The following detailed description is provided for the additional convenience of the reader.

Definitions and basic techniques

As used in this disclosure, "TRRE activity" refers to the ability of a composition to cleave and release TNF receptors from the surface of cells expressing them. A preferred assay is cleavage from transfected COS-1 cells, as described in Example 1. However, TRRE activity can be measured on any cells that bear TNF receptors of the 55 kDa or 75 kDa size. Other features of the TRRE enzyme obtained from PMA induction of THP-1 cells (exemplified in Example 2) need not be a property of the TRRE activity measured in the assay.

Unit activity of TRRE is defined as 1 pg of soluble p75 TNF-R released from cell surface in a standard assay, after correction for spontaneous release. The measurement of TRRE activity is explained further in Example 1.

A "TRRE modulator" is a compound that has the property of either increasing or decreasing TRRE activity for processing TNF on the surface of

cells. Those that increase TRRE activity may be referred to as TRRE promoters, and those that decrease TRRE activity may be referred to as TRRE inhibitors. TRRE promoters include compounds that have proteolytic activity for TNF-R, and compounds that augment the activity of TNF-R proteases. The nine polynucleotide clones described in Example 5, and their protein products, are exemplary TRRE promoters. Inhibitors of TRRE activity can be obtained using the screening assays described below.

The term "polynucleotide" refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, (mRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide refers interchangeably to double-and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form, and each of two complementary single-stranded forms known or predicted to make up the double-stranded form

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. Hybridization reactions can be performed under conditions of different "stringency". Relevant conditions include temperature, ionic strength, and the presence of additional solutes in the reaction mixture such as formamide. Conditions of increasing stringency are 30°C. in 10X SSC (0.15M NaC1, 15 mM citrate buffer); 40°C. in 6X SSC; 50°C. in 6.X SSC 60°C. in 6X SSC, or at about 40°C. in 0.5X SSC, or at about 30°C. in 6.X. SSC containing 50% formamide. SDS and a source of fragmented DNA (such as salmon sperm) are typically also present during hybridization. Higher

stringency requires higher minimum complementarity between hybridizing elements for a stable hybridization complex to form. See "Molecular Cloning: A Laboratory Manual", Second Edition (Sambrook, Fritsch & Maniatis, 1989).

It is understood that purine and pyrimidine nitrogenous bases with similar structures can be functionally equivalent in terms of Watson-Crick base-pairing; and the inter-substitution of like nitrogenous bases, particularly uracil and thymine, or the modification of nitrogenous bases, such as by methylation, does not constitute a material substitution.

The percentage of sequence identity for polynucleotides or polypeptides is calculated by aligning the sequences being compared, and then counting the number of shared residues at each aligned position. No penalty is imposed for the presence of insertions or deletions, but are permitted only where required to accommodate an obviously increased number of amino acid residues in one of the sequences being aligned. When one of the sequences being compared is indicated as being "consecutive", then no gaps are permitted in that sequence during the comparison. The percentage identity is given in terms of residues in the test sequence that are identical to residues in the comparison or reference sequence.

As used herein, "expression" of a polynucleotide refers to the production of an RNA transcript. Subsequent translation into protein or other effector compounds may also occur, but is not required unless specified.

"Genetic alteration" refers to a process wherein a genetic element is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alternation may be effected, for example, by transducing a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. It is preferable that the

genetic alteration is inheritable by progeny of the cell, but this is not generally required unless specified.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

A "fusion polypeptide" is a polypeptide comprising regions in a different position in the sequence than occurs in nature. The regions can normally exist in separate proteins and are brought together in the fusion polypeptide; they can normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide; or they can be synthetically arranged. A "functionally equivalent fragment" of a polypeptide varies from the native sequence by addition, deletion, or substitution of amino acid residues, or any combination thereof, while preserving a functional property of the fragment relevant to the context in which it is being used. Fusion peptides and functionally equivalent fragments are included in the definition of polypeptides used in this disclosure.

It is understood that the folding and the biological function of proteins can accommodate insertions, deletions, and substitutions in the amino acid sequence. Some amino acid substitutions are more easily tolerated. For example, substitution of an amino acid with hydrophobic side chains, aromatic side chains, polar side chains, side chains with a positive or negative charge, or side chains comprising two or fewer carbon atoms, by another amino acid with a side chain of like properties can occur without disturbing the essential identity of the two sequences. Methods for determining homologous regions and scoring the degree of homology are described in Altschul et al. *Bull. Math. Bio.* 48:603-616, 1986; and Henikoff et al. *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992. Substitutions that preserve the functionality of the polypeptide, or confer a new

and beneficial property (such as enhanced activity, stability, or decreased immunogenicity) are especially preferred.

An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact antibodies, but also antibody equivalents that include at least one antigen combining site of the desired specificity. These include but are not limited to enzymatic or recombinantly produced fragments antibody, fusion proteins, humanized antibodies, single chain variable regions, diabodies, and antibody chains that undergo antigen-induced assembly.

An "isolated" polynucleotide, polypeptide, protein, antibody, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially obtained from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis or recombinant expression.

A "host cell" is a cell which has been genetically altered, or is capable of being transformed, by administration of an exogenous polynucleotide.

The term "clinical sample" encompasses a variety of sample types obtained from a subject and useful in an in vitro procedure, such as a diagnostic test. The definition encompasses solid tissue samples obtained as a surgical removal, a pathology specimen, or a biopsy specimen, cells obtained from a

clinical subject or their progeny obtained from culture, liquid samples such as blood, serum, plasma, spinal fluid, and urine, and any fractions or extracts of such samples that contain a potential indication of the disease.

Unless otherwise indicated, the practice of the invention will employ conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, within the skill of the art. Such techniques are explained in the standard literature, such as: "Molecular Cloning: A Laboratory Manual", Second Edition (Sambrook, Fritsch & Maniatis, 1989), "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984), "Animal Cell Culture" (R. I. Freshney, ed., 1987); the series "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (D. M. Weir & C. C. Blackwell, Eds.), "Gene Transfer Vectors for Mammalian Cells" (J. M. Miller & M. P. Calos, eds., 1987), "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987); and "Current Protocols in Immunology" (J. E. Coligan et al., eds., 1991). The reader may also choose to refer to a previous patent application relating to TRRE, International Patent Application WO 98020140.

For purposes of prosecution in the U.S., and in other jurisdictions where allowed, all patents, patent applications, articles and publications indicated anywhere in this disclosure are hereby incorporated herein by reference in their entirety.

Polynucleotides

Polynucleotides of this invention can be prepared by any suitable technique in the art. Using the data provided in this disclosure, sequences of less than ~50 base pairs are conveniently prepared by chemical synthesis, either through a commercial service or by a known synthetic method, such as the triester method or the phosphite method. A preferred method is solid phase synthesis using mononucleoside phosphoramidite coupling units (Hirose et al., *Tetra. Lett.* 19:2449-2452, 1978; U.S. Patent No. 4,415,732).

For use in antisense therapy, polynucleotides can be prepared by chemistry that produce more stable in pharmaceutical preparations. Non-limiting

examples include thiol-derivatized nucleosides (U.S. Patent 5,578,718), and oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825).

Polynucleotides of this invention can also be obtained by PCR amplification of a template with the desired sequence. Oligonucleotide primers spanning the desired sequence are annealed to the template, elongated by a DNA polymerase, and then melted at higher temperature so that the template and elongated oligonucleotides dissociate. The cycle is repeated until the desired amount of amplified polynucleotide is obtained (U.S. Patent Nos. 4,683,195 and 4,683,202). Suitable templates include the Jurkat T cell library and other human or animal expression libraries that contain TRRE modulator encoding sequences. The Jurkat T cell library is available from the American Type Culture Collection, 10801 University Blvd., Manassas VA 20110, U.S.A. (ATCC #TIB-152). Mutations and other adaptations can be performed during amplification by designing suitable primers, or can be incorporated afterwards by genetic splicing.

Production scale amounts of large polynucleotides are most conveniently obtained by inserting the desired sequence into a suitable cloning vector and reproducing the clone. Techniques for nucleotide cloning are given in Sambrook, Fritsch & Maniatis (supra) and in U.S. Patent No. 5,552,524. Exemplary cloning and expression methods are illustrated in Example 6.

Preferred polynucleotide sequences are 50%, 70%, 80% , 90%, or 100% identical to one of the sequences exemplified in this disclosure; in order if increasing preference. The length of consecutive residues in the identical or homologous sequence compared with the exemplary sequence can be about 15, 30, 50, 75, 100, 200 or 500 residues in order of increasing preference, up to the length of the entire clone. Nucleotide changes that cause a conservative substitution or retain the function of the encoded polypeptide (in terms of hybridization properties or what is encoded) are especially preferred substitutions.

The polynucleotides of this can be used to measure altered TRRE activity in a cell or tissue sample. This involves contacting the sample with the polynucleotide under conditions that permit the polynucleotide to hybridize specifically with nucleic acid that encodes a modulator of TRRE activity, if present in the sample, and determining polynucleotide that has hybridized as a result of step a). Specificity of the test can be provided in one of several ways. One method involves the use of a specific probe — a polynucleotide of this invention with a sequence long enough and of sufficient identity to the sequence being detected, so that it binds the target and not other nucleic acid that might be present in the sample. The probe is typically labeled (either directly or through a secondary reagent) so that it can be subsequently detected. Suitable labels include ^{32}P and ^{33}P, chemiluminescent and fluorescent reagents. After the hybridization reaction, unreacted probe is washed away so that the amount of hybridized probe can be determined. Signal can be amplified using branched probes (U.S. Patent No. 5,124,246). In another method, the polynucleotide is a primer for a PCR reaction. Specificity is provided by the ability of the paired probes to amplify the sequence of interest. After a suitable number of PCR cycles, the amount of amplification product present correlates with the amount of target sequence originally present in the sample.

Such tests are useful both in research, and in the diagnosis or assessment of a disease condition. For example, TNF activity plays a role in eliminating tumor cells (Example 4), and a cancer may evade the elimination process by activating TRRE activity in the diseased tissue. Hence, under some conditions, high expression of TRRE modulators may correlate with progression of cancer. Diagnostic tests are also of use in monitoring therapy, such as when gene therapy is performed to increase TRRE activity.

Polynucleotides of this invention can also be used for production of polypeptides and the preparation of medicaments, as explained below.

Polypeptides

Short polypeptides of this invention can be prepared by solid-phase chemical synthesis. The principles of solid phase chemical synthesis can be found in Dugas & Penney, Bioorganic Chemistry, Springer-Verlag NY pp 54-92 (1981), and U.S. Patent No. 4,493,795. Automated solid-phase peptide synthesis can be performed using devices such as a PE-Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City CA).

Longer polypeptides are conveniently obtained by expression cloning. A polynucleotide encoding the desired polypeptide is operably linked to control elements for transcription and translation, and then transfected into a suitable host cell. Expression may be effected in procaryotes such as E. coli (ATCC Accession No. 31446 or 27325), eukaryotic microorganisms such as the yeast Saccharomyces cerevisiae, or higher eukaryotes, such as insect or mammalian cells. A number of expression systems are described in U.S. Patent No. 5 ,552,524. Expression cloning is available from such commercial services as Lark Technologies, Houston TX. The production of protein from 4 exemplary clones of this invention in insect cells is illustrated in Example 6. The protein is purified from the producing host cell by standard methods in protein chemistry, such as affinity chromatography and HPLC. Expression products are optionally produced with a sequence tag to facilitate affinity purification, which can subsequently be removed.

Preferred sequences are 40%, 60%, 80% , 90%, or 100% identical to one of the sequences exemplified in this disclosure; in order if increasing preference. The length of the identical or homologous sequence compared with the native human polynucleotide can be about 7, 10, 15, 20, 30, 50 or 100 residues in order of increasing preference, up to the length of the entire encoding region.

Polypeptides can be tested for an ability to modulate TRRE in a TNF-R cleavage assay. The polypeptide is contacted with the receptor (preferably expressed on the surface of a cell, such as a C75 cell), and the ability of the polypeptide to increase or decrease receptor cleavage and release is

determined. Cleavage of TNF-R by exemplary polypeptides of this invention is illustrated in Example 7.

Polypeptides of this invention can be used as immunogens for raising antibody. Large proteins will raise a cocktail of antibodies, while short peptide fragments will raise antibodies against small region of the intact protein. Antibody clones can be mapped for protein binding site by producing short overlapping peptides of about 10 amino acids in length. Overlapping peptides can be prepared on a nylon membrane support by standard F-Moc chemistry, using a SPOTS™ kit from Genosys according to manufacturer's directions.

Polypeptides of this invention can also be used to affect TNF signal transduction, as explained below.

Antibodies

Polyclonal antibodies can be prepared by injecting a vertebrate with a polypeptide of this invention in an immunogenic form. Immunogenicity of a polypeptide can be enhanced by linking to a carrier such as KLH, or combining with an adjuvant, such as Freund's adjuvant. Typically, a priming injection is followed by a booster injection is after about 4 weeks, and antiserum is harvested a week later. Unwanted activity cross-reacting with other antigens, if present, can be removed, for example, by running the preparation over adsorbants made of those antigens attached to a solid phase, and collecting the unbound fraction. If desired, the specific antibody activity can be further purified by a combination of techniques, which may include protein, A chromatography, ammonium sulfate precipitation, ion exchange chromatography, HPLC, and immunoaffinity chromatography using the immunizing polypeptide coupled to a solid support. Antibody fragments and other derivatives can be prepared by standard immunochemical methods, such as subjecting the antibody to cleavage with enzymes such as papain or pepsin.

Production of monoclonal antibodies is described in such standard references as Harrow & Lane (1988), U.S. Patent Nos. 4,491,632, 4,472,500 and 4,444,887, and *Methods in Enzymology* 73B:3 (1981). Briefly, a mammal is

immunized, and antibody-producing cells (usually splenocytes) are harvested. Cells are immortalized by fusion with a non-producing myeloma, transfecting with Epstein Barr Virus, or transforming with oncogenic DNA. The treated cells are cloned and cultured, and the clones are selected that produce antibody of the desired specificity.

Other methods of obtaining specific antibody molecules (optimally in the form of single-chain variable regions) involve contacting a library of immunocompetent cells or viral particles with the target antigen, and growing out positively selected clones. Immunocompetent phage can be constructed to express immunoglobulin variable region segments on their surface. See Marks et al., *New Eng. J. Med.* 335:730, 1996, International Patent Applications WO 9413804, WO 9201047, WO 90 02809, and McGuiness et al., *Nature Biotechnol.* 14:1449, 1996.

The antibodies of this invention are can be used in immunoassays for TRRE modulators. General techniques of immunoassay can be found in "The Immunoassay Handbook", Stockton Press NY, 1994; and "Methods of Immunological Analysis", Weinheim: VCH Verlags gesellschaft mbH, 1993). The antibody is combined with a test sample under conditions where the antibody will bind specifically to any modulator that might be present, but not any other proteins liable to be in the sample. The complex formed can be measured in situ (U.S. Patent Nos. 4,208,479 and 4,708,929), or by physically separating it from unreacted reagents (U.S. Patent No. 3,646,346). Separation assays typically involve labeled TRRE reagent (competition assay), or labeled antibody (sandwich assay) to facilitate detection and quantitation of the complex. Suitable labels are radioisotopes such as ^{125}I, enzymes such as β-galactosidase, and fluorescent labels such as fluorescein. Antibodies of this invention can also be used to detect TRRE modulators in fixed tissue sections by immunohistology. The antibody is contacted with the tissue, unreacted antibody is washed away, and then bound antibody is detected — typically using a labeled anti-immunoglobulin reagent. Immunohistology will show not only whether the modulator is present, but where it is located in the tissue.

Detection of TRRE modulators is of interest for research purposes, and for clinical use. As indicated earlier, high expression of TRRE modulators may correlate with progression of cancer. Diagnostic tests are also of use in monitoring TRRE modulators that are administered in the course of therapy.

Antibodies of this invention can also be used for preparation of medicaments. Antibodies with therapeutic potential include those that affect TRRE activity — either by promoting clearance of a TRRE modulator, or by blocking its physiological action. Antibodies can be screened for desirable activity according to assays described in the next section.

Screening assays

This invention provides a number of screening methods for selecting and developing products that modulate TRRE, and thus affect TNF signal transduction.

One screening method is for polynucleotides that have an ability to modulate TRRE activity. To do this screening, cells are obtained that express both TRRE and the TNF receptor. Suitable cell lines can be constructed from any cell that expresses a level of functional TRRE activity. These cells are identifiable by testing culture supernatant for an ability to release membrane-bound TNF-R. The level of TRRE expression should be moderate, so that an increase in activity can be detected. The cells can then be genetically altered to express either p55 or p75 TNF-R, illustrated in Example 1. Exemplary is the C75R line: COS-1 cells genetically altered to express the 75 kDa form of the TNF-R. Release of TNF-R from the cell can be measured either by testing residual binding of labeled TNF ligand to the cell, or by immunoassay of the supernatant for released receptor (Example 1).

The screening assay is conducted by contacting the cells expressing TRRE and TNF-R with the polynucleotides to be screened. The effect of the polynucleotide on the enzymatic release of TNF-R from the cell is determined, and polynucleotides with desirable activity (either promoting or inhibiting TRRE activity) are selected. In a variation of this method, cells expressing TRRE

activity but not TNF-R (such as untransfected COS-1 cells) are contacted with the test polynucleotide. Then the culture medium is collected, and used to assay for TRRE activity using a second cell expressing TNF-R (such as C75 cells).

This type of screening assay is useful for the selection of polynucleotides from an expression library believed to contain encoding sequences for TRRE modulators. The Jurkat cell expression library (ATCC Accession No. TIB-152) is exemplary. Other cells from which suitable libraries can be constructed are those known to express high levels of TRRE, especially after PMA stimulation, such as THP-1, U-937, HL-60, ME-180, MRC-5, Raji, K-562, and normal human monocytes. The screening involves expressing DNA from the library in the selected cell line being used for screening. Wells with the desired activity are selected, and the DNA is recovered, optionally after replication or cloning of the cells. Repeat cycles of functional screening and selection can lead to identification of new polynucleotide clones that promote or inhibit TRRE activity. This is illustrated below in Example 5. Further experiments can be performed on the selected polynucleotides to determine it modulates TRRE activity inside the cell, or through the action of a protein product. A long open reading frame suggests a role for a protein product, and examination of the amino acid sequence for a signal peptide and a membrane spanning region can help determine whether the protein is secreted from the cell or expressed in the surface membrane.

This type of screening is also useful for further development of the polynucleotides of this invention. For example, expression constructs can be developed that encode functional peptide fragments, fusion proteins, and other variants. The minimum size of polynucleotide sequence that still encodes TRRE modulation activity can be determined by removing part of the sequence and then using the screening assay to determine whether the activity is still present. Mutated and extended sequences can be tested in the same way.

This type of screening assay is also useful for developing compounds that affect TRRE activity by interfering with mRNA that encode a TRRE modulator. Of particular interest are ribozymes and antisense oligonucleotides. Ribozymes

are endoribonucleases that catalyze cleavage of RNA at a specific site. They comprise a polynucleotide sequence that is complementary to the cleavage site on the target, and additional sequence that provide the tertiary structure to effect the cleavage. Construction of ribozymes is described in U.S. Patent Nos. 4,987,071 and 5,591,610. Antisense oligonucleotides that bind mRNA comprise a short sequence complementary to the mRNA (typically 8-25 bases in length). Preferred chemistry for constructing antisense oligonucleotides is outlined in an earlier section. Specificity is provided both by the complementary sequence, and by features of the chemical structure. Antisense molecules that inhibit expression of cell surface receptors are described in U.S. Patent Nos. 5,135,917 and 5,789,573. Screening involves contacting the cell expressing TRRE activity and TNF-R with the compound and determining the effect on receptor release. Ribozymes and antisense molecules effective in altering expression of a TRRE promoter would decrease TNF-R release. Ribozymes and antisense molecules effective in altering expression of a TRRE inhibitor would increase TNF-R release.

Another screening method described in this disclosure is for testing the ability of polypeptides to modulate TRRE activity (Example 7). Cells expressing both TNF-R and a moderate level of TRRE activity are contacted with the test polypeptides, and the rate of receptor release is compared with the rate of spontaneous release. An increased rate of release indicates that the polypeptide is a TRRE promoter, while a decreased rate indicates that the polypeptide is a TRRE inhibitor. This assay can be used to test the activity of new polypeptides, and develop variants of polypeptides already known to modulate TRRE. The minimum size of polypeptide sequence that still encodes TRRE modulation activity can be determined by making a smaller fragment of the polypeptide and then using the screening assay to determine whether the activity is still present. Mutated and extended sequences can be tested in the same way.

Another screening method embodied in this invention is a method for screening substances that interfere with the action of a TRRE modulator at the protein level. The method involves incubating cells expressing TNF receptor

(such as C75R cells) with a polypeptide of this invention having TNF promoting activity. There are two options for supplying the TRRE modulator in this assay. In one option, the polypeptide is added to the medium of the cells as a reagent, along with the substance to be tested. In another option, the cells are genetically altered to express the TRRE modulator at a high level, and the assay requires only that the test substance be contacted with the cells. This option allows for high throughput screening of a number of test compounds.

Either way, the rate of receptor release is compared in the presence and absence of the test substance, to identify compounds that enhance or diminish TRRE activity. Parallel experiments should be conducted in which the activity of the substance on receptor shedding is tested in the absence of added polypeptide (using cells that don't express the polypeptide). This will determine whether the activity of the test substance occurs via an effect on the TRRE promoter being added, or through some other mechanism.

This type of screening assay is useful for identifying antibodies that affect the activity of a TRRE modulator. Antibodies are raised against a TRRE modulator as described in the previous section. If the antibody decreases TRRE activity in the screening assay, then it has therapeutic potential to lower TRRE activity in vivo. Screening of monoclonal antibodies using this assay can also help identify binding or catalytic sites in the polypeptide.

This type of screening assay is also useful for high throughput screening of small molecule compounds that have the ability to affect the level of TNF receptors on a cell, by way of its influence on a TRRE modulator. Small molecule compounds that have the desired activity are often preferred for pharmaceutical compositions, because they are often more stable and less expensive to produce.

Medicaments and their use

As described earlier, a utility of certain products embodied in this invention is to affect signal transduction from cytokines (particularly TNF). Products that promote TRRE activity have the effect of decreasing TNF receptors on the

surface of cells, which would decrease signal transduction from TNF. Conversely, products that inhibit TRRE activity prevent cleavage of TNF receptors, increasing signal transduction.

The ability to affect TNF signal transduction is of considerable interest in the management of clinical conditions in which TNF signaling contributes to the pathology of the condition. Such conditions include:

- Heart failure. IL-1ß and TNF are believed to be central mediators for perpetuating the inflammatory process, recruiting and activating inflammatory cells. The inflammation depress cardiac function in congestive heart failure, transplant rejection, myocarditis, sepsis, and burn shock.
- Cachexia. The general weight loss and wasting occurring in the course of chronic diseases, such as cancer. TNF is believed to affect appetite, energy expenditure, and metabolic rate.
- Crohn's disease. The inflammatory process mediated by TNF leads to thickening of the intestinal wall, ensuing from lymphedema and lymphocytic infiltration.
- Endotoxic shock. The shock induced by release of endotoxins from gram-negative bacteria, such as E. coli, involves TNF-mediated inflammation
- Arthritis. TNF promotes expression of nitric oxide synthetase, believed to be involved in disease pathogenesis.

Other conditions of interest are multiple sclerosis, sepsis, inflammation brought on by microbe infection, and diseases that have an autoimmune etiology, such as Type I Diabetes.

Polypeptides of this invention that promote TRRE activity can be administered with the objective of decreasing or normalizing TNF signal transduction. For example, in congestive heart failure or Crohn's disease, the polypeptide is given at regular intervals to lessen the inflammatory sequelae. The treatment is optionally in combination with other agents that affect TNF

signal transduction (such as antibodies to TNF or receptor antagonists) or that lessen the extent of inflammation in other ways.

Polynucleotides of this invention can also be used to promote TRRE activity by gene therapy. The encoding sequence is operably linked to control elements for transcription and translation in human cells. It is then provided in a form that will promote entry and expression of the encoding sequence in cells at the disease site. Forms suitable for local injection include naked DNA, polynucleotides packaged with cationic lipids, and polynucleotides in the form of viral vectors (such as adenovirus and AAV constructs). Methods of gene therapy known to the practitioner skilled in the art will include those outlined in U.S. Patent Nos. 5,399,346, 5,827,703, and 5,866,696.

The ability to affect TNF signal transduction is also of interest where TNF is thought to play a beneficial role in resolving the disease. In particular, TNF plays a beneficial role in the necrotizing of solid tumors. Accordingly, products of this invention can be administered to cancer patients to inhibit TRRE activity, thereby increasing TNF signal transduction and improve the beneficial effect.

Embodiments of the invention that inhibit TRRE activity include antisense polynucleotides. A method of conferring long-standing inhibitory activity is to administer antisense gene therapy. A genetic construct is designed that will express RNA inside the cell which in turn will decrease the transcription of the target gene (U.S. Patent No. 5,759,829). In humans, a more frequent form of antisense therapy is to administer the effector antisense molecule directly, in the form of a short stable polynucleotide fragment that is complementary to a segment of the target mRNA (U.S Patent Nos. 5,135,917 and 5,789,573) — in this case, the transcript that encodes the TRRE modulator. Another embodiment of the invention that inhibits TRRE are ribozymes, constructed as described in an earlier section. The function of ribozymes in inhibiting mRNA translation is described in U.S. Patent Nos. 4,987,071 and 5,591,610.

Once a product of this invention is found to have suitable TRRE modulation activity in the in vitro assays described in this disclosure, it is preferable to also test its effectiveness in an animal model of a TNF mediated

disease process. Example 3 describes an LPS model for sepsis that can be used to test promoters of TRRE activity. Example 4 describes a tumor necrosis model, in which TRRE inhibitors could be tested for an ability to enhance necrotizing activity. Those skilled in the art will know of other animal models suitable for testing effects on TNF signal transduction or inflammation. Other illustrations are the cardiac ischemia reperfusion models of Weyrich et al. (*J. Clin. Invest.* 91:2620, 1993) and Garcia-Criado et al. (*J. Am. Coll. Surg.* 181:327, 1995); the pulmonary ischemia reperfusion model of Steinberg et al. (*J. Heart Lung Transplant.* 13:306, 1994), the lung inflammation model of International Patent Application WO 9635418; the bacterial peritonitis model of Sharar et al. (*J. Immunol.* 151:4982, 1993), the colitis model of Meenan et al. (*Scand. J. Gastroenterol.* 31:786, 1996), and the diabetes model of von Herrath et al. (*J. Clin. Invest.* 98:1324, 1996). Models for septic shock are described in Mack et al. *J. Surg. Res.* 69:399, 1997; and Seljelid et al. *Scand. J. Immunol.* 45:683-7.

For use as an active ingredient in a pharmaceutical preparation, a polypeptide, polynucleotide, or antibody of this invention is generally purified away from other reactive or potentially immunogenic components present in the mixture in which they are prepared. Typically, each active ingredient is provided in at least about 90% homogeneity, and more preferably 95% or 99% homogeneity, as determined by functional assay, chromatography, or SDS polyacrylamide gel electrophoresis. The active ingredient is then compounded into a medicament in accordance with generally accepted procedures for the preparation of pharmaceutical preparations, such as described in *Remington's Pharmaceutical Sciences 18th Edition* (1990), E.W. Martin ed., Mack Publishing Co., PA. Steps in the compounding of the medicament depend in part on the intended use and mode of administration, and may include sterilizing, mixing with appropriate non-toxic and non-interfering excipients and carriers, dividing into dose units, and enclosing in a delivery device. The medicament will typically be packaged with information about its intended use.

Mode of administration will depend on the nature of the condition being treated. For conditions that are expected to require moderate dosing and that are at well perfused sites (such as cardiac failure), systemic administration is acceptable. For example, the medicament may be formulated for intravenous administration, intramuscular injection, or absorption sublingually or intranasally. Where it is possible to administer the active ingredient locally, this is usually preferred. Local administration will both enhance the concentration of the active ingredient at the disease site, and minimize effects on TNF receptors on other tissues not involved in the disease process. Conditions that lend themselves to administration directly at the disease site include cancer and rheumatoid arthritis. Solid tumors can be injected directly when close to the skin, or when they can be reached by an endoscopic procedure. Active ingredients can also be administered to a tumor site during surgical resection, being implanted in a gelatinous matrix or in a suitable membrane such as Gliadel® (Guilford Sciences). Where direct administration is not possible, the administration may be given through an arteriole leading to the disease site. Alternatively, the pharmaceutical composition may be formulated to enhance accumulation of the active ingredient at the disease site. For example, the active ingredient can be encapsulated in a liposome or other matrix structure that displays an antibody or ligand capable of binding a cell surface protein on the target cell. Suitable targeting agents include antibodies against cancer antigens, ligands for tissue-specific receptors (e.g., serotonin for pulmonary targeting). For compositions that decrease TNF signal transduction, an appropriate targeting molecule may be the TNF ligand, since the target tissue may likely display an unusually high density of the TNF receptor.

Effective amounts of the compositions of the present invention are those that alter TRRE activity by at least about 10%, typically by at least about 25%, more preferably by about 50% or 75%. Where near complete ablation of TRRE activity is desirable, preferred compositions decrease TRRE activity by at least 90%. Where increase of TRRE activity is desirable, preferred compositions increase TRRE activity by at least 2-fold. A minimum effective amount of the

active compound will depend on the disease being treated, which of the TRRE modulators is selected for use, and whether the administration will be systemic or local. For systemic administration, an effective amount of activity will generally be an amount of the TRRE modulator that can cause a change in the enzyme activity by 100 to 50,000 Units — typically about 10,000 Units. The mass amount of protein, nucleic acid, or antibody is chosen accordingly, based on the specific activity of the active compound in Units per gram.

The following examples provided as a further guide to the practitioner, and are not intended to limit the invention in any way.

EXAMPLES

Example 1: Assay system for TRRE activity.

This Example illustrates an assay system that measures TRRE activity on the human TNF-R in its native conformation in the cell surface membrane

Membrane-associated TNF-R was chosen as the substrate, as having microenvironment similar to that of the substrate for TRRE in vivo. Membrane-associated TNF-R also requires more specific activity, which would differentiate less-specific proteases. Cells expressing an elevated level of the p75 form of TNF-R were constructed by cDNA transfection into monkey COS-1 cells which express little TNF-R of either the 75 kDa or 55 kDa size.

The procedure for constructing these cells was as follows: cDNA of human p75 TNF-R was cloned from a λgt10 cDNA library derived from human monocytic U-937 cells (Clontech Laboratories, Palo Alto, CA). The first 300 bp on both 5' and 3' ends of the cloned fragment was sequenced and compared to the reported cDNA sequence of human p75 TNF-R. The cloned sequence was a 2.3 kb fragment covering positions 58-2380 of the reported p75 TNF-R sequence, which encompasses the full length of the p75 TNF-R-coding sequence from positions 90-1475. The 2.3 kb p75 TNF-R cDNA was then subcloned into the multiple cloning site of the pCDNA3 eukaryotic expression vector. The

orientation of the p75 TNF-R cDNA was verified by restriction endonuclease mapping.

Figure 1 illustrates the final 7.7 kb construct, pCDTR2. It carries the neomycin-resistance gene for the selection of transfected cells in G418, and the expression of the p75 TNF-R is driven by the cytomegalovirus promoter. The pCDTR2 was then transfected into monkey kidney COS-1 cells (ATCC CRL-1650) using the calcium phosphate-DNA precipitation method. The selected clone in G418 medium was identified and subcultured. This clone was given the designation **C75R.**

To determine the level of p75 TNF-R expression on C75R cells, 2 x 10^5 cells/well were plated into a 24-well culture plate and incubated for 12 to 16 hours in 5% CO_2 at 37°C. They were then incubated with 2-30 ng ^{125}I human recombinant TNF (radiolabeled using the chloramine T method) in the presence or absence of 100-fold excess of unlabeled human TNF at 4°C for 2 h. After three washes with ice-cold PBS, cells were lysed with 0.1N NaOH and bound radioactivity was determined in a Pharmacia Clinigamma counter (Uppsala, Sweden).

Figure 2 shows the results obtained. C75R had a very high level of specific binding of radiolabeled ^{125}I-TNF, while parental COS-1 cells did not. The number of TNF-R expressed on C75R was determined to be 60,000-70,000 receptors per cell by Scatchard analysis (Figure 2, inset). The Kd value calculated was 5.6 x 10^{-10} M. This Kd value was in close agreement to the values previously reported for native p75 TNF-R.

TRRE was obtained by PHA stimulation of THP-1 cells (WO 9802140). THP-1 cells (ATCC 45503) growing in logarithmic phase were collected and resuspended to 1x10^6 cells/ml of RPMI-1640 supplemented with 1% FCS and incubated with 10^{-6} M PMA for 30 min in 5% CO_2 at 37 °C. The cells were collected and washed once with serum-free medium to remove PMA and resuspended in the same volume of RPMI-1640 with 1% FCS. After 2 hours incubation in 5% CO_2 at 37°C, the cell suspension was collected, centrifuged, and the cell-free supernatant was collected as the source of TRRE.

In order to measure the effect of TRRE on membrane-bound TNF-R in the COS-1 cell constructs, the following experiment was performed. C75R cells were seeded at a density of 2 x 10^5 cells/well in a 24-well cell culture plate and incubated for 12 to 16 hours at 37°C in 5% CO_2. The medium in the wells was aspirated, replaced with fresh medium alone or with TRRE medium, and incubated for 30 min at 37°C, The medium was then replaced with fresh medium containing 30 ng/ml ^{125}I-labeled TNF. After 2 hours at 4°C, the cells were lysed with 0.1 N NaOH and the level of bound radioactivity was measured. The level of specific binding of C75R by ^{125}I-TNF was significantly decreased after incubation with TRRE. The radioactive count was 1,393 cpm on the cells incubated with TRRE compared to 10,567 cpm on the cells not treated with TRRE, a loss of 87% of binding capacity.

In order to determine the size of the p75 TNF-R cleared from C75R by TRRE, the following experiment was performed. 15 x 10^6 C75R cells were seeded in a 150 mm cell culture plate and incubated at 37°C in 5% CO_2 for 12 to 16 hours. TRRE medium was incubated with C75R cells in the 150 mm plate for 30 min and the resulting supernatant was collected and centrifuged. The concentrated sample was applied to 10% acrylamide SDS-PAGE and electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon). Immunostaining resulted in a single band of 40 kDa, similar to the size found in biological fluids. Thus, transfected COS-1 cells expressed high levels of human p75 TNF-R in a form similar to native TNF-R.

The following assay method was adopted for routine measurement of TRRE activity. C75R cells and COS-1 cells were seeded into 24-well culture plates at a density of 2.5 x 10^5 cells/ml/well and incubated overnight (for 12 to 16 hours) in 5% CO_2 at 37°C. After aspirating the medium in the well, 300 µl of TRRE medium was incubated in each well of both the C75R and COS-1 plates for 30 min in 5% CO_2 at 37°C (corresponding to A and C mentioned below, respectively). Simultaneously, C75R cells in 24-well plates were also incubated with 300 µl of fresh medium or buffer . The supernatants were collected,

centrifuged, and then assayed for the concentration of soluble p75 TNF-R by ELISA.

ELISA assay for released TNF-R (WO 9802140) was performed as follows: Polyclonal antibodies to human p75 TNF-R were generated by immunization of New Zealand white female rabbits (Yamamoto et al. *Cell. Immunol.* 38:403-416, 1978). The IgG fraction of the immunized rabbit serum was purified using a protein G (Pharmacia Fine Chemicals, Uppsala, Sweden) affinity column (Ey et al. (1978) *Immunochemistry* 15:429-436, 1978). The IgG fraction was then labeled with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO) (Tijssen and Kurstok, *Anal. Biochem.* 136:451-457, 1984). In the first step of the assay, 5 μg of unlabeled IgG in 100 μl of 0.05 M carbonate buffer (pH 9.6) was bound to a 96-well ELISA microplate (Corning, Corning, NY) by overnight incubation at 4°C. Individual wells were washed three times with 300 μl of 0.2% Tween-20 in phosphate buffered saline (PBS). The 100 μl of samples and recombinant receptor standards were added to each well and incubated at 37°C for 1 to 2 hours. The wells were then washed in the same manner, 100 μl of horseradish peroxidase-labeled IgG added and incubated for 1 hour at 37 °C. The wells were washed once more and the color was developed for 20 minutes (min) at room temperature with the substrates ABTS (Pierce, Rockford, IL) and 30% H_2O_2 (Fisher Scientific, Fair Lawn, NJ). Color development was measured at 405 nm.

When C75R cells were incubated with TRRE medium, soluble p75 TNF-R was released into the supernatant which was measurable by ELISA. The amount of receptors released corresponded to the amount of TRRE added There was also a level of spontaneous TNF-R release in C75R cells incubated with just medium alone. It is hypothesized that this is due to an endogenous source of proteolytic enzyme, a homolog of the human TRRE of monkey origin.

The following calculations were performed. A = (amount of soluble p75 TNF-R in a C75R plate treated with the TRRE containing sample); i.e. the total amount of sTNF-R in a C75R plate. B = (amount of soluble p75 TNF-R spontaneously released in a C75R plate treated with only medium or buffer

containing the same reagent as the corresponding samples but without exogenous TRRE); i.e. the spontaneous release of sTNF-R from C75R cells. C = (amount of soluble p75 TNF-R in a COS-1 plate treated with the TRRE sample or the background level of soluble p75 TNF-R released by THP-1.); i.e. the degraded value of transferred (pre-existing) sTNF-R in the TRRE sample during 30 min incubation in a COS-1 plate. This corresponds to the background level of sTNF-R degraded in a C75R plate. The net release of soluble p75 TNF-R produced only by TRRE activity existing in the initial sample is calculated as follows: (Net release of soluble p75 TNF-R only by TRRE) = A - B - C.

Unit activity of TRRE was defined as follows: 1 pg of soluble p75 TNF-R net release (A-B-C) in the course of the assay is one unit (U) of TRRE activity.

Using this assay, the time course of receptor shedding by TRRE was measured in the following experiment. TRRE-medium was incubated with C75R and COS-1 cells for varying lengths of time. The supernatants were then collected and assayed for the level of soluble p75 TNF-R by ELISA and the net TRRE activity was calculated. Detectable levels of soluble receptor were released by TRRE within 5 min and increased up to 30 min. Longer incubation times showed that the level of TRRE remained relatively constant after 30 min, presumably from the depletion of substrates. Therefore, 30 min was determined to be the optimal incubation time.

The induction patterns of TRRE and known MMPs by PMA stimulation are quite different. In order to induce MMPs, monocytic U-937 cells, fibrosarcoma HT-1080 cells, or peritoneal exudate macrophages (PEM) usually have to be stimulated for one to three days with LPS or PMA. On the other hand, as compared with this prolonged induction, TRRE is released very quickly in culture supernatant following 30 min of PMA-stimulation. The hypothesis that TRRE and sTNF-R form a complex *in vitro* was confirmed by the experiment that 25% TRRE activity was recovered from soluble p75 TNF-R affinity column. This means that free TRRE has the ability to bind to its catalytic product, sTNF-R. The remaining 75% which did not combine to the affinity column may already be

bound to sTNF-R or may not have enough affinity to bind to sTNF-R even though it is in a free form.

Example 2: Characterization of TRRE obtained from THP-1 cells .

TRRE obtained by PHA stimulation of THP-1 cells was partially purified from the culture medium (WO 9802140). First, protein from the medium was concentrated by 100% saturated ammonium sulfate precipitation at 4°C. The precipitate was pelleted by centrifugation at 10,000 x g for 30 min and resuspended in PBS in approximately twice the volume of the pellet. This solution was then dialyzed at 4°C against 10 mM Tris-HCl, 60 mM NaCl, pH 7.0. This sample was loaded on an anion-exchange chromatography, Diethylaminoethyl (DEAE)-Sephadex A-25 column (Pharmacia Biotech) (2.5 x 10 cm) previously equilibrated with 50 mM Tris-HCl, 60 mM NaCl, pH 8.0. TRRE was then eluted with an ionic strength linear gradient of 60 to 250 mM NaCl, 50 mM Tris-HCl, pH 8.0. Each fraction was measured for absorbance at 280 nm and assayed for TRRE activity. The DEAE fraction with the highest specific activity (the highest value of TRRE units/A280) was pooled and used in the characterizations of TRRE described in this example.

In the next experiment, the substrate specificity of the enzyme was elucidated using immunohistochemical techniques. Fluorescein isothiocyanate (FITC)-conjugated anti-CD54, FITC-conjugated goat anti-rabbit and mouse antibodies, mouse monoclonal anti-CD30, anti-CD11b and anti-IL-1R (Serotec, Washington D.C.) were used. Rabbit polyclonal anti-p55 and p75 TNF-R were obtained according to Yamamoto et al. (1978) *Cell Immunol.* 38:403–416. THP-1 cells were treated for 30 min with 1,000 and/or 5,000 U/ml of TRRE eluted from the DEAE-Sephadex column, and then transferred to 12 x 75 mm polystyrene tubes (Fischer Scientific, Pittsburgh, PA) at 1 x 10^5 cells/100µl/tube. The cells were then pelleted by centrifugation at 350 x g for 5 min at 4°C and stained directly with 10µl FITC-conjugated anti-CD54 (diluted in cold PBS/0.5% sodium aside), indirectly with FITC-conjugated anti-mouse antibody after treatment of

mouse monoclonal anti-CD11b, IL-1R and CD30 and also indirectly with FITC-conjugated anti-rabbit antibody after treatment of rabbit polyclonal anti-p55 and p75 TNF-R.

THP-1 cells stained with each of the antibodies without treatment of TRRE were used as negative controls. The tubes were incubated for 45 min at 4°C, agitated every 15 min, washed twice with PBS/2% FCS, repelleted and then resuspended in 200µl of 1% paraformaldehyde. These labeled THP-1 cells were analyzed using a fluorescence activated cell sorter (FACS) (Becton-Dickinson, San Jose, CA) with a 15 mW argon laser with an excitation of 488 nm. Fluorescent signals were gated on the basis of forward and right angle light scattering to eliminate dead cells and aggregates from analysis. Gated signals (10^4) were detected at 585 BP filter and analyzed using Lysis II software. Values were expressed as percentage of positive cells, which was calculated by dividing mean channel fluorescence intensity (MFI) of stained THP-1 cells treated with TRRE by the MFI of the cells without TRRE treatment (negative control cells).

To test the *in vitro* TNF cytolytic assay by TRRE treatment the L929 cytolytic assay was performed according to the method described by Gatanaga et al. (1990b). Briefly, L929 cells, an adherent murine fibroblast cell line, were plated (70,000 cells/0.1ml/well in a 96-well plate) overnight. Monolayered L929 cells were pretreated for 30 min with 100, 500 or 2,500 U/ml of partially-purified TRRE and then exposed to serial dilutions of recombinant human TNF for 1 hour. After washing the plate with RPMI-1640 with 10% FCS to remove the TRRE and TNF, the cells were incubated for 18 hours in RPMI-1640 with 10% FCS containing 1 µg/ml actinomycin D at 37°C in 5% CO_2. Culture supernatants were then aspirated and 50 µl of 1% crystal violet solution was added to each well. The plates were incubated for 15 min at room temperature. After the plates were washed with tap water and air-dried, the cells stained with crystal violet were lysed by 100 µl per well of 100 mM HCl in methanol. The absorbance at

550 nm was measured using an EAR 400 AT plate reader (SLT-Labinstruments, Salzburg, Austria).

To investigate whether TRRE also truncates the ~55 kDa size of TNF-R, partially-purified TRRE was applied to THP-1 cells which express low levels of both p55 and p75 TNF-R (approximately 1,500 receptors/cell by Scatchard analysis). TRRE eluate from the DEAE-Sephadex column was added to THP-1 cells (5 x 10^6 cells/ml) at a final TRRE concentration of 1,000 U/ml for 30 min. The concentration of soluble p55 and p75 TNF-R in that supernatant was measured by soluble p55 and p75 TNF-R ELISA. TRRE was found to truncate both human p55 and p75 TNF-R on THP-1 cells and released 2,382 and 1,662 pg/ml soluble p55 and p75 TNF-R, respectively.

Therefore,TRRE obtained by PHA stimulation of THP-1 cells is capable of enzymatically cleaving and releasing human p75 TNF-R on C75R cells, and both human p55 and p75 TNF-R on THP-1 cells.

Partial inhibition of TRRE activity was obtained by chelating agents such as 1,10-phenanthroline, EDTA and EGTA (% TRRE activity remaining were 41%, 67% and 73%, respectively, at 2 mM concentration). On the other hand, serine protease inhibitors such as PMSF, AEBSF and 3,4-DCI, and serine and cysteine protease inhibitors such as TLCK and TPCK had no effect on the inhibition of TRRE. TRRE was slightly activated in the presence of Mn^{2+}, Ca^{2+}, Mg^{2+}, and Co^{2+} (% TRRE activities remaining were 157%, 151%, 127%, and 123%, respectively), whereas partial inhibition occurred in the presence of Zn^{2+} and Cu^{2+} (% TRRE activities remaining were 23% and 47%, respectively) (WO 9802140).

TRRE fractions from the most active DEAE fraction (60 mM to 250 mM NaCl) can be purified further. In one method (WO 9802140), the fractions were concentrated to 500 µL with a Centriprep-10 filter (10,000 MW cut-off membrane) (Amicon). This concentrated sample was applied to 6% PAGE under non-denaturing native conditions. The gel was sliced horizontally into 5 mm strips and each was eluted into 1 ml PBS. The eluates were then tested according to the assay (Example 1) for TRRE activity.

Example 3: TRRE activity alleviates septic shock

The following protocol was used to test the effects of TRRE in preventing mortality in a model for septic shock. Mice were injected with lethal or sublethal levels of LPS, and then with a control buffer or TRRE. Samples of peripheral blood were then collected at intervals to establish if TRRE blocked TNF-induced production of other cytokines in the bloodstream. Animals were assessed for the ability of TRRE to block the clinical effects of shock, and then euthanized and tissues examined by histopathological methods.

Details were as follows: adult Balb/c mice, were placed in a restraining device and injected intravenously via the tail vein with a 0.1 ml solution containing 10 ng to 10 mg of LPS in phosphate buffer saline (PBS). These levels of LPS induce mild to lethal levels of shock in this strain of mice. Shock results from changes in vascular permeability, fluid loss, and dehydration, and is often accompanied by symptoms including lethargy, a hunched, stationary position, rumpled fur, cessation of eating, cyanosis, and, in serious cases, death within 12 to 24 hours. Control mice received an injection of PBS. Different amounts (2,000 or 4,000 U) of purified human TRRE were injected IV in a 0.1 ml volume within an hour prior to or after LPS injection. Serum (0.1 ml) was collected with a 27 gauge needle and 1 ml syringe IV from the tail vein at 30, 60 and 90 minutes after LPS injection. This serum was heparinized and stored frozen at –20°C. Samples from multiple experiments were tested by ELISA for the presence of sTNF-R, TNF, IL-8 and IL-6. Animals were monitored over the next 12 hours for the clinical effects of shock. Selected animals were euthanized at periods from 3 to 12 hours after treatment, autopsied and various organs and tissues fixed in formalin, imbedded in paraffin, sectioned and stained by hematoxalin-eosin (H and E). Tissue sections were subjected to histopathologic and immunopathologic examination.

Figure 3 shows the results obtained. (◆) LPS alone; (■) LPS plus control buffer; (●) LPS plus TRRE (2,000 U); (▲) LPS plus TRRE (4,000 U).

Mice injected with LPS alone or LPS and a control buffer died shortly after injection. 50% of the test animals were dead after 8 hours (LPS) or 9 hours (LPS plus control buffer), and 100% of the animals were dead at 15 hours. In contrast, animals treated with TRRE obtained as described in Example 1 did much better. When injections of LPS were accompanied by injections of a 2,000 U of TRRE, death was delayed and death rates were lower. Only 40% of the animals were dead at 24 hours. When 4,000 U of TRRE was injected along with LPS, all of the animals had survived at 24 hours. Thus, TRRE is able to counteract the mortality induced by LPS in test animals.

Example 4: TRRE activity decreases tumor necrotizing activity

The following protocol was followed to test the effects of TRRE on tumor necrosis in test animals in which tumors were produced, and in which TNF was subsequently injected.

On Day 0, cutaneous Meth A tumors were produced on the abdominal wall of fifteen BALB/c mice by intradermal injection of 2×20^5 Meth A tumor cells. On Day 7, the mice were divided into three groups of five mice each and treated as follows:

- Group 1: Injected intravenously with TNF (1 μg/mouse).
- Group 2: Injected intravenously with TNF (1 μg/mouse) and injected intratumorally with TRRE obtained as in Example 1 (400 units/mouse, 6, 12 hours after TNF injection).
- Group 3: Injected intravenously with TNF (1 μg/mouse) and injected intratumorally with control medium (6, 12 hours after TNF injection).

On Day 8, tumor necrosis was measured with the following results: Group 1: 100% of necrosis (5/5); Group 2: 20% (1/5); Group 3: 80% (4/5). Injections of TRRE greatly reduced the ability of TNF to induce necrosis in Meth A tumors in BALB/c mice.

Since adding TRRE activity ablates the beneficial necrotizing activity of TNF, blocking endogenous TRRE activity would promote the beneficial effects of TNF.

Example 5: Nine new polynucleotide clones that affect TRRE activity

A number of cells have been found to express high levels of TRRE activity, especially after PMA stimulation. These include the cell lines designated THP-1, U-937, HL-60, ME-180, MRC-5, Raji, K-562. Jurkat cells have a high TRRE activity (850 TRRE U/mL at 10^{-2} PMA). In this experiment, the expression library of the Jurkat T cell (ATCC #TIB-152) was obtained and used to obtain 9 polynucleotide clones that augment TRRE activity.

Selection of expression sequences in the library was done by repeated cycles of transfection into COS-1 cells, followed by assaying of the supernatant as in Example 1 for the presence of activity cleaving and releasing the TNF receptor. Standard techniques were used in the genetic manipulation. Briefly, the DNA of 10^6 Jurkat cells was extracted using an InVitrogen plasmid extraction kit according to manufacturer's directions. cDNA was inserted in the ZAP Express™/Eco*RI* vector (cat. no. 938201, Stratagene, La Jolla CA. The library was divided into 48 groups of DNA and transformed into COS-1 cells using the CaCl transfection method. Once the cells were grown out, the TRRE assay was performed, and five positive groups were selected. DNA from each of these five groups was obtained, and transfected into *E. coli*, with 15 plates per group. DNA was prepared from these cells and then transfected into COS-1 cells once more. The cells were grown out, and TRRE activity was tested again. Two positive groups were selected and transfected into *E. coli,* yielding 98 colonies. DNA was prepared from 96 of these colonies and transfected into COS-1 cells. The TRRE activity was performed again, and nine clones were found to substantially increase TRRE activity in the assay. These clones were designated 2-8, 2-9, 2-14, 2-15, P2-2, P2-10, P2-13, P2-14, and P2-15.

Figure 4 is a bar graph showing the TRRE activity observed when the 9 clones were tested with C75 cells in the standard assay (Example 1).

These nine clones were then sequenced according to the following procedure:

1. Plasmid DNA was prepared using a modified alkaline lysis procedure.
2. DNA sequencing was performed using DyeDeoxy termination reactions (ABI). Base-specific fluorescent dyes were used as labels.
3. Sequencing reactions were analyzed on 5.75% Long Ranger™ gels by an ABI 373A-S or on 5.0% Long Ranger™ gels by an ABI 377 automated sequencer.
4. Subsequent data analysis was performed using Sequencher™ 3.0 software.

Standard primers T7X, T3X, -40, -48 Reverse, and BK Reverse (BKR) were used in sequencing reactions. For each clone, several additional internal sequencing primers (listed below) were synthesized.

NCBI BLAST (Basic Local Alignment Search Tool) sequence analysis (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410) was performed to determine if other sequences were significantly similar to these sequences. Both the DNA sequences of the clones and the corresponding ORFs (if any) were compared to sequences available in databases.

The following clones were obtained and sequenced:

TABLE 1: DNA sequences affecting TRRE activity					
Clone	Sequence Designation	SEQ ID NO:	Approx. Length (bp)	Expression Designation	Related sequences (potential homology)
2-9	AIM2	1	4,047		—
2-8	AIM3T3 (partial sequence)	2	739		*M. musculus* 45S pre-rRNA gene
	AIM3T7 (partial sequence)	3	233		
2-14	AIM4	4	2,998	Mey3	human arfaptin 2 and others (see below)
2-15	AIM5	5	4,152		—
P2-2	AIM6	6	3,117	Mey5	—
P2-10	AIM7	7	3,306	Mey6	Human Insulin-like Growth factor II Receptor
P1-13	AIM8	8	4,218		—
P2-14	AIM9	9	1,187	Mey8	—
P2-15	AIM10	10	3,306		E1b-55kDa-associated protein

Clone 2-9 (AIM2): The internal primers used for sequencing are shown in SEQ. ID NOS:11-38. The sequence of AIM2 is presented in SEQ ID NO:1. The complementary strand of the AIM2 sequence is SEQ ID NO:147. The longest open reading frame (ORF) in the AIM2 sequence is 474 AA long and represented in SEQ ID NO:148.

Clone 2-8 (AIM3): Two partial sequences of length 739 and 233 were obtained and designated AIM3T3 and AIM3T7. The internal primers used for sequencing are shown in SEQ. ID NOS:39-46. The sequences of AIM3T3 and

AIM3T7 are presented in SEQ ID NOs:2 and 3, respectively. The BLAST search revealed that the AIM3T3 sequence may be homologous to the mouse (*M. musculus*) 28S ribosomal RNA (Hassouna et al. *Nucleic Acids Res.* 12:3563-3583, 1984) and the *M. musculus* 45S pre-rRNA genes (Accession No. X82564. The complementary sequence of the AIM3T3 sequence showed 99% similarity over 408 bp beginning with nt 221 of SEQ ID NO:2 to the former and 97% similarity over the same span to the latter.

Clone 2-14 (AIM4). The internal primers used for sequencing are shown in SEQ. ID NOS:14-65. The sequence of AIM4 is presented in SEQ ID NO:4. The complementary strand of the AIM4 sequence is SEQ ID NO:149. The longest ORF in the AIM4 sequence is 236 AA long and represented in SEQ ID NO:150. AIM4 has significant alignments to human sequences arfaptin 2, ADE2H1 mRNA showing homologies to SAICAR synthetase, polypyrimidine tract binding protein (heterogeneous nuclear ribonucleoprotein I) mRNA, several PTB genes for polypirimidine tract binding proteins, mRNA for por1 protein. Human arfaptin 2 is a putative target protein of ADP-ribosylation factor that interacts with RAC1 by binding directly to it. RAC1 is involved in membrane ruffling. Arfaptin 2 has possible transmembrane segments, potential CK2 phosphorylation sites, PKC phosphorylation site and RGD cell attachment sequence.

Clone 2-15 (AIM5): The internal primers used for sequencing are shown in SEQ. ID NOS:66-80. The sequence of AIM5 is presented in SEQ ID NO:5. The BLAST search revealed that the AIM5 sequence displays some similarity to Human Initiation Factor 5A (eIF-5A) Koettnitz et al. (1995) *Gene* 159:283-284, 1995 and Human Initiation Factor 4D (eIF 4D) Smit-McBride et al. (1989) *J. Biol. Chem.* 264:1578-1583, 1989.

Clone P2-2 (AIM6): The internal primers used for sequencing are shown in SEQ. ID NOS:81-93. The sequence of AIM6 is presented in SEQ ID NO:6. The longest ORF in the AIM6 sequence is 1038 AA long and represented in SEQ ID NO:151.

Clone P2-10 (AIM7): The internal primers used for sequencing are shown in SEQ. ID NOS:94-106. The sequence of AIM7 is presented as SEQ ID NO:7.

The longest ORF in the AIM7 sequence is 849 AA long and represented in SEQ ID NO:152. The BLAST search revealed that this clone may be related to the Human Insulin-like Growth Factor II Receptor (Morgan et al. *Nature* 329:301-307, 1987 or the Human Cation-Independent Mannose 6-Phosphate Receptor mRNA (Oshima et al. *J. Biol. Chem.* 263:2553-2562, 1988). The AIM7 sequence showed roughly 99% identity to both sequences over 2520 nucleotides beginning with nt 12 of SEQ ID NO:7 and 99% similarity to the latter over the same span.

Clone P2-13 (AIM8): The internal primers used for sequencing are shown in SEQ. ID NOS:107-118. The sequence of AIM8 is presented as SEQ ID NO:8. The longest ORF in the AIM8 sequence is 852 AA long and represented in SEQ ID NO:153.

Clone P2-14 (AIM9): The internal primers used for sequencing are shown in SEQ. ID NOS:119-124. The sequence of AIM9 is presented as SEQ ID NO:9. The longest ORF was about 149 amino acids in length.

Clone P2-15 (AIM10): The internal primers used for sequencing are shown in SEQ. ID NOS:125-146. The sequence of AIM10 is presented as SEQ ID NO:10. The longest ORF in the AIM10 sequence is 693 AA long and represented in SEQ ID NO:154. Sequence 10 on BLASTN search of non-redundant databases at NCBI aligns with Human mRNA for E1b-55kDa-associated protein, locus HSA7509 (Accession AJ007509, NID g3319955).

Clonal DNA may be directly injected into test animals in order to test the ability of these nucleic acids to induce TRRE activity, counteract septic shock and/or affect tumor necrosis, as is described in detail in Examples 3 and 4. Alternatively, proteins or RNA can be generated from the clonal DNA for similar testing.

Example 6: Expression of newly obtained clones

Example 5 describes 9 new clones which enhance TRRE activity in a cell surface assay system. The clones were obtained in the pBK-CMB Phagmid vector .

The following work was done on contract through the commercial laboratory Lark Technologies, Houston, TX. The clones were removed from shuttle vectors and inserted into expression vectors in the following manner. Recombinant plasmid (pBK-CMV containing insert) was digested with appropriate restriction enzyme(s) such as Spe I, Xba I, EcoR I or others, as appropriate. The Baculovirus Transfer Vector (pAcGHLT-A Baculovirus Transfer Vector, PharMingen, San Diego, CA, Cat. No. 21460P) was also cut with appropriate restriction enzyme(s) within or near the multiple cloning site to receive the insert removed from the shuttle vector.

The fragment of interest being sublconed was isolated from the digest using Low-Melting agarose electrophoresis and purified from the gel using a Qiaquick Gel Extraction Kit following Lark SOP MB 020602. If necessary, the receiving vector was treated with alkaline phosphatase according to Lark SOP MB 090201. The fragment was ligated into the chosen site of the vector pAcGHLT-A. The recombinant plasmid was transformed into *E. coli* XL1 Blue MRF' cells and the transformed bacterial cells were selected on LB agar plates containing ampicillin (100μg/ml). Ampicillin resistant colonies were picked and grown on LB broth containing ampicillin for plasmid preparation.

Plasmid DNA was prepared using Alkaline Minilysate Procedure (Lark SOP MB 010802 and digested with appropriate restriction enzyme(s). Selected subclones were confirmed to be of the correct size. Sublcones were digested with other appropriate restriction enzyme(s) to ascertain correct orientation of the insert by confirming presence of fragments of proper size(s). A subclone was grown in 100 ml of LB broth containing ampicillin (100μg/ml) and the plasmid DNA prepared using Qiagen Midi Plasmid Preparation Kit (Lark SOP MB 011001). The DNA concentration was determined by measuring the absorbance at 260 nm and the DNA sample was verified to be originated from correct subclone by restriction digestion.

Thus were produced the expression constructs for Mey3, Mey5, Mey6, Mey8 now with the coding sequence of interest fused to GST gene with polyhistitidine tag, protein kinase A site and thrombin cleavage site. The GST

gene and now the fusion protein are under the polyhedrin promotor. PharMingen (San Diego, CA) incorporated the vector with insert into functional baculovirus particles by co-inserting the transfer vector (pAcGHLT) into susceptible insect cell line S along with linearized virus DNA (PharMingen, San Diego, CA, BaculoGold viral DNA, Cat. No. 21100D). The functional virus particles were grown again on the insect cells to generate a high titer stock. Protein production was then done by infecting a large culture of cells in Tini cell. The cells were harvested when the protein yield reached a maximum and before the virus killed the cells. Fusion proteins were collected on a glutatione-agarose column, washed and released with glutathionine.

Proteins collected from the affinity column were quantified by measuring OD_{280} and were assayed on gels using SDS-PAGE and Western blotting with labeled anti-GST (PharMingen, San Diego, CA, mAbGST Cat. No. 21441A) to confirm that all the bands present included the GST portion.

Four of the ten sequences have been cloned, expressed in bacculovirus infected insect cells, and then purified.

TABLE 2: Expressed protein from Jurkat library clones

Name	Sequence in insert	Amount of protein (mg/mL)
Mey3	AIM4	4.7, 5.0
Mey5	AIM6	1.36, 1.50
Mey6	AIM7	0.33
Mey8	AIM9	1.53

Gels indicated the presence of the GST protein in addition to larger proteins that were also positive with the anti-GST antibody in Western analyses. Mey3 repeatedly exhibited the presence of proteins around 32kDa, 56kDa, bands around 60-70kDa and another larger than 70kDa. Mey5 consistently had proteins migrating as approximately 34kDa, 38kDa, 58kDa, around 60-70kDa, and others larger than 70kDa. Mey6 had protein bands around 34kDa, 56kDa,

58kDa, and bands around 60-70kDa. Mey8 had protein bands around 36kDa, 58kDa and bands around 60-70kDa. All of the indicated bands were positive for GST. The bands may represent the desired fusion protein or degradation/cleavage product generated during growth and purification.

Example 7: Assay of expression products for effect on TNF-R cleaving activity

The following method was used to measure TRRE activity of Mey 3, 5, 6 and 8. C75R cells and COS-1 cells were seeded into 24-well culture plates at a density of 2.5 x 10^5 cells/ml/well and incubated overnight (for 12 to16 hours) in 5% CO_2 at 37°C. After aspirating the medium in the well, 300µl of 1 ug of Mey 3, 5 and 8 were incubated in each well of both the C75R and COS-1 plates for 30 min in 5% CO_2 at 37°C (corresponding to A and C mentioned below, respectively). Simultaneously, C75R cells in 24-well plates were also incubated with 300µl of fresh medium or buffer (corresponding to B mentioned below). The supernatants were collected, centrifuged, and then assayed for the concentration of soluble p75 TNF-R by ELISA as described in Example 1.

The following results were obtained:

TABLE 3: Enzymatic activity of expressed clones

Clone No.	TNF-receptor releasing activity U/mg
Mey-3	341
Mey-5	671
Mey-6	452
Mey-8	191

Example 8: Effectiveness of expression products in treating septic shock

The protocol outlined in Example 3 was used to test the effects of the expression products from the new clones in preventing mortality in the septic shock model.

Different amounts of recombinant Mey 3, 5, and 8 (10 – 100 ug/mouse) were injected i.v. in a 0.05 ml volume within an hour prior to or after injection of a lethal dose of LPS. Serum (0.1ml) was collected using a 27 gauge needle and 1 ml syringe from the tail vein at 30, 60 and 90 minutes after LPS injection. This serum was heparinized and stored frozen at -20°C. Samples from multiple experiments were tested by ELISA for the presence of solubilized TNR-R, the TNR ligand, IL-8, and IL-6. Animals were monitored over the next 12 hours for the clinical effects of shock. Selected animals were euthanized from 3 to 12 hours after treatment, autopsied and various organs and tissues fixed in formalin, imbedded in paraffin, sectioned and stained by hematoxalin-eosin (H and E). Tissue sections were subjected to histopathologic and immunopathologic examination.

Figure 5 shows the results obtained. (◆) saline; (■) BSA; (△) Mey-3 (100 μg); (X) Mey-3 (10 μg); (*) Mey-5 (10 μg); (●) Mey-8 (10 μg).

Mice injected with LPS alone or LPS, a control buffer or control protein (BSA) died rapidly. All of the animals in this group were dead at 24 hours. In contrast, when injections of LPS were accompanied by injections of a 10 – 100 ug of Mey 3, 5 and 8, death was delayed and death rates were lower. None of the animal were dead at 24 hours that had been treated with Mey 3 and Mey 5. Only 66 % of the animals were dead at 24 hours that had been treated with Mey 8. Thus, Mey 3, 5 and 8 were able to counteract the mortality induced by LPS in test animals.

CLAIMS

What is claimed as the invention is:

1. An isolated polynucleotide comprising a nucleotide sequence with the following properties:
   a) the sequence is expressed at the mRNA level in Jurkat T cells;
   b) when COS-1 cells expressing TNF receptor are genetically altered to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor.

2. The polynucleotide of claim 1, wherein the nucleotide sequence is contained in a sequence selected from the group consisting of
   a) SEQ. ID NO:1;
   b) SEQ. ID NO:2 or SEQ. ID NO:3;
   c) SEQ. ID NO:4;
   d) SEQ. ID NO:5;
   e) SEQ. ID NO:6;
   f) SEQ. ID NO:7;
   g) SEQ. ID NO:8;
   h) SEQ. ID NO:9; and
   i) SEQ. ID NO:10.

3. An isolated polynucleotide comprising at least 30 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to any of claims 1-3

4. An isolated polynucleotide comprising a linear sequence of at least 50 consecutive nucleotides at least 90% identical to a sequence contained in said nucleotide sequence of the polynucleotide of claim 1.

5. An isolated polynucleotide of at least 50 nucleotides capable of hybridizing specifically to said nucleotide sequence of a polynucleotide according to any of claims 1-3 at 68°C in 0.5 M phosphate buffer pH 7, 7% SDS, and 100 μg/mL salmon sperm DNA, followed by washing in a buffer containing 3X SSC.

6. An antisense polynucleotide or ribozyme comprising at least 10 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to claim 1 or 2, which inhibits the expression of a TRRE modulator.

7. An isolated polypeptide comprising an amino acid sequence encoded by a polynucleotide according to any of claims 1-5.

8. The polypeptide of claim 7, selected from the group consisting of SEQ. ID NOS: 147-158.

9. An isolated polypeptide, comprising at least 10 consecutive residues in said amino acid sequence of a polypeptide according to claim 7 or 8.

10. An isolated polypeptide, comprising at least 15 consecutive amino acids which are at least 80% identical to a sequence contained in said amino acid sequence of the polypeptide according to claim 7 or 8.

11. The polypeptide of claim 7-11, which when incubated with COS-1 cells expressing TNF receptor, promotes enzymatic cleavage and release of the receptor.

12. The polypeptide of claims 7-11, which either:
    a) lacks a membrane spanning sequence; or

b) is produced by a process comprising recombinant expression in a host cell followed by purification of the polypeptide from medium in which the cell is cultured.

13. A method of producing the polypeptide according to any of claims 7 to 11, comprising the steps of:

a) culturing host cells genetically altered to express the polynucleotide according to claim 3; and subsequently

b) purifying the polypeptide from the cells.

14. The method according to claim 13, comprising harvesting culture medium following step a); and purifying the polypeptide from the culture medium by a process comprising affinity chromatography.

15. An isolated polynucleotide encoding the polypeptide of claim 8 or 9.

16. An isolated antibody specific for a polypeptide according any of claims 7-11.

17. A method for producing the antibody according to claim 16, comprising immunizing a mammal or contacting an immunocompetent cell or particle with a polypeptide according to claim 9 or 10.

18. An assay method of determining altered TRRE activity in a cell or tissue sample, comprising the steps of:

a) contacting the sample with the polynucleotide of claim 4 or 5 under conditions that permit the polynucleotide to hybridize specifically with nucleic acid that encodes a modulator of TRRE activity, if present in the sample; and

b) determining polynucleotide that has hybridized as a result of step a), as a measure of altered TRRE activity in the sample.

19. An assay method for determining altered expression of a modulator of TRRE activity in a cell or tissue sample, comprising the steps of:
a) contacting the sample with the antibody of claim 16 under conditions that permit the antibody to bind the modulator if present in the sample, thereby forming an antibody-antigen complex; and
b) determining complex formed in step a), as a measure of the modulator.

20. A method for assessing a disease condition associated with altered TRRE activity in a subject, comprising determining altered TRRE activity in the sample from the subject according to claim 18, or determining altered expression of a TRRE modulator according to claim 19, and then correlating the extent of alteration with the disease condition.

21. A method for decreasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polypeptide according to any of claims 7-8 and 11-12, or with a polynucleotide according to any of claims 1-3 and 15.

22. A method for increasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polynucleotide according to claim 6, or with an antibody according to claim 16.

23. The method according to claim 21 or claim 22, wherein the cytokine is TNF.

24. A method for screening polynucleotides for an ability to modulate TRRE activity, comprising the steps of:
a) providing cells that express both TRRE and the TNF-receptor;
b) genetically altering the cells with the polynucleotides to be screened;
c) cloning the cells genetically altered in step b); and

d) identifying clones that enzymatically release the receptor at an altered rate.

25. A method for screening substances for an ability to affect TRRE activity, comprising the steps of:

a) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the presence of the substance;

b) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the absence of the substance;

c) measuring any TNF receptor released from the cells in steps a) and b); and

d) correlating an increase or decrease of the receptor released in step a) relative to that in step b) with an ability of the substance to enhance or diminish TRRE activity.

26. Use of a polypeptide according to any of claims 7-8 or 11-12, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

27. Use of a polynucleotide according to any of claims 1-3, 6, or 15 in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

28. Use of an antibody according to claim 16, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

29. Use of a polypeptide according to any of claims 7-8 and 11-12, a polynucleotide according to any of claims 1-3 and 15 or an antibody according to claim 16, in the preparation of a medicament for treatment of a

disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis.

30. A method of treating cancer in a subject, comprising increasing signal transduction from TNF into cells at the site of the cancer in the subject according to claim 22 or 23.

31. A method of treating a disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis, comprising decreasing signal transduction from TNF into cells at the site of the disease in the subject according to claim 21 or 23.

32. The method of claim 31, comprising administering to the subject an effective amount of the polypeptide of any of claims 7-8 or 11-12.

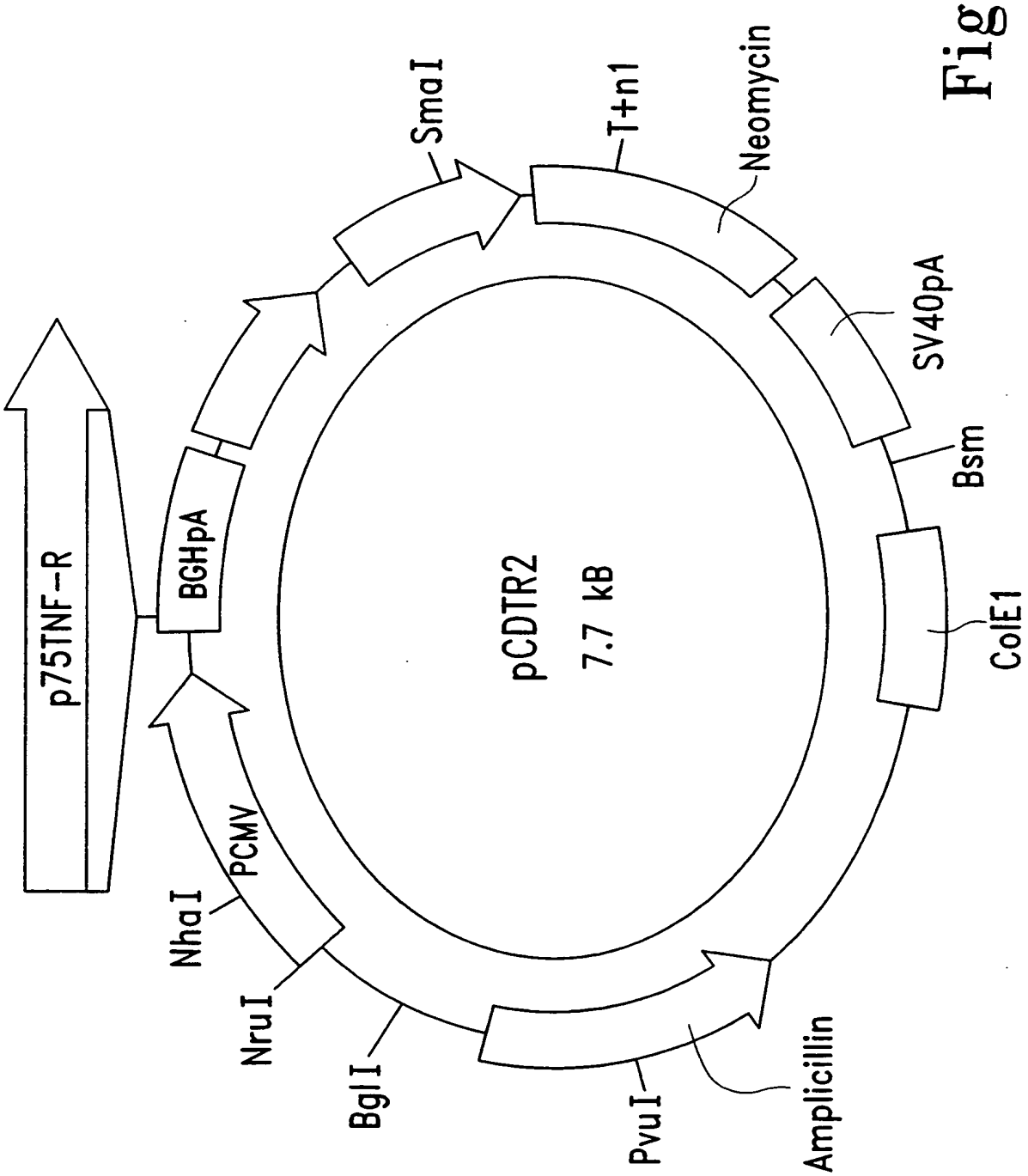
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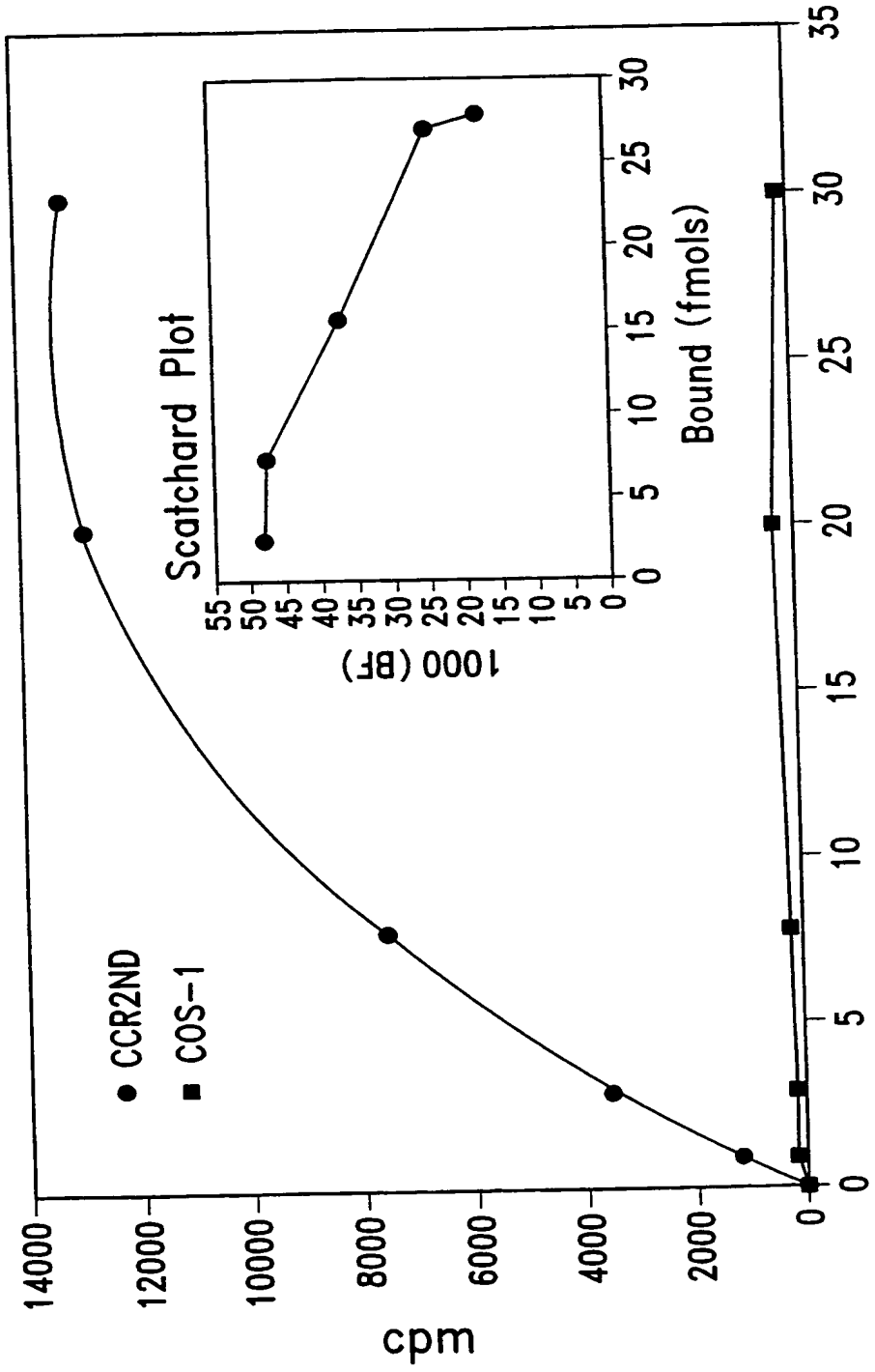
Fig. 1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35
Scatchard Plot
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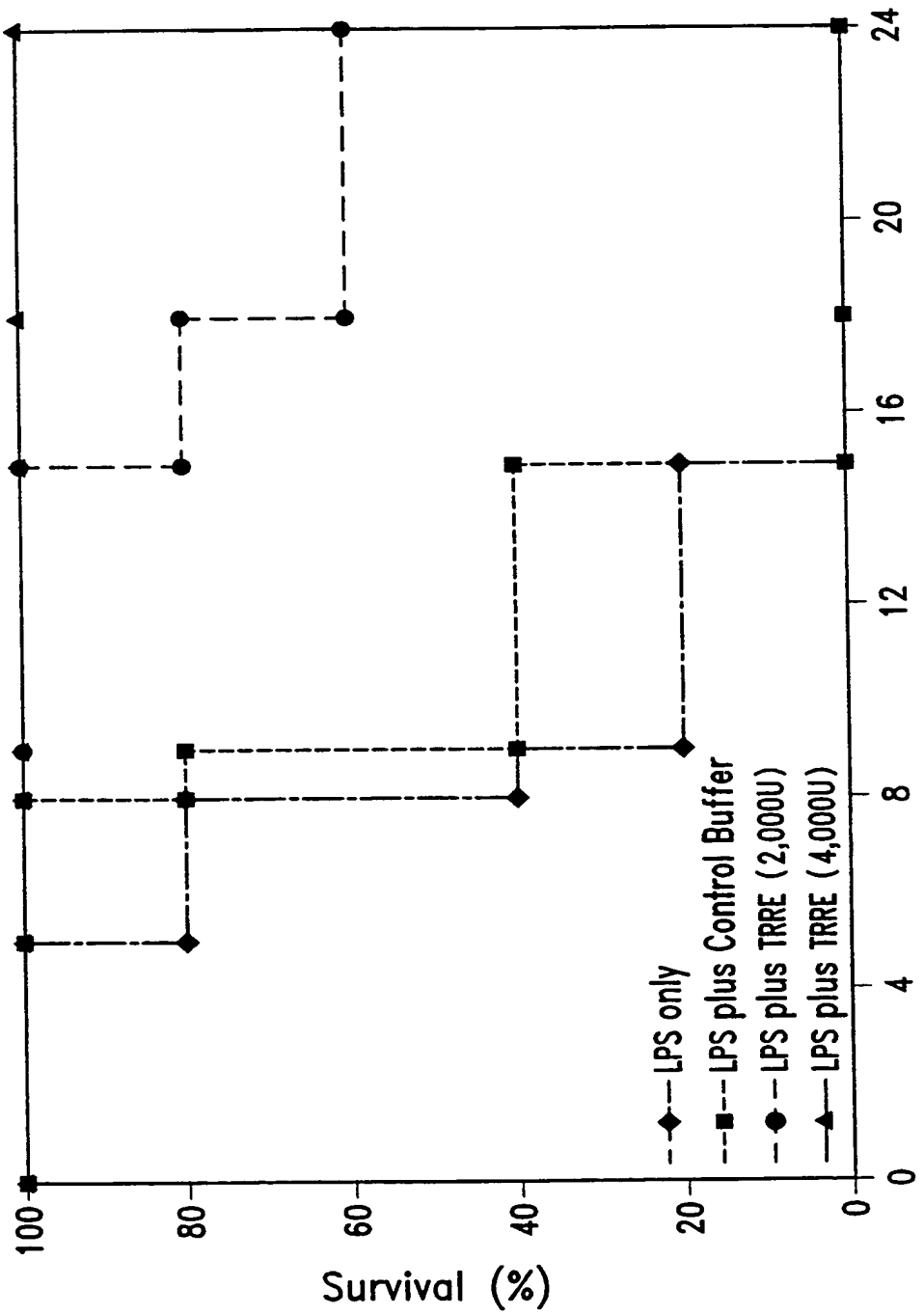
Fig. 2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60
40
20
0
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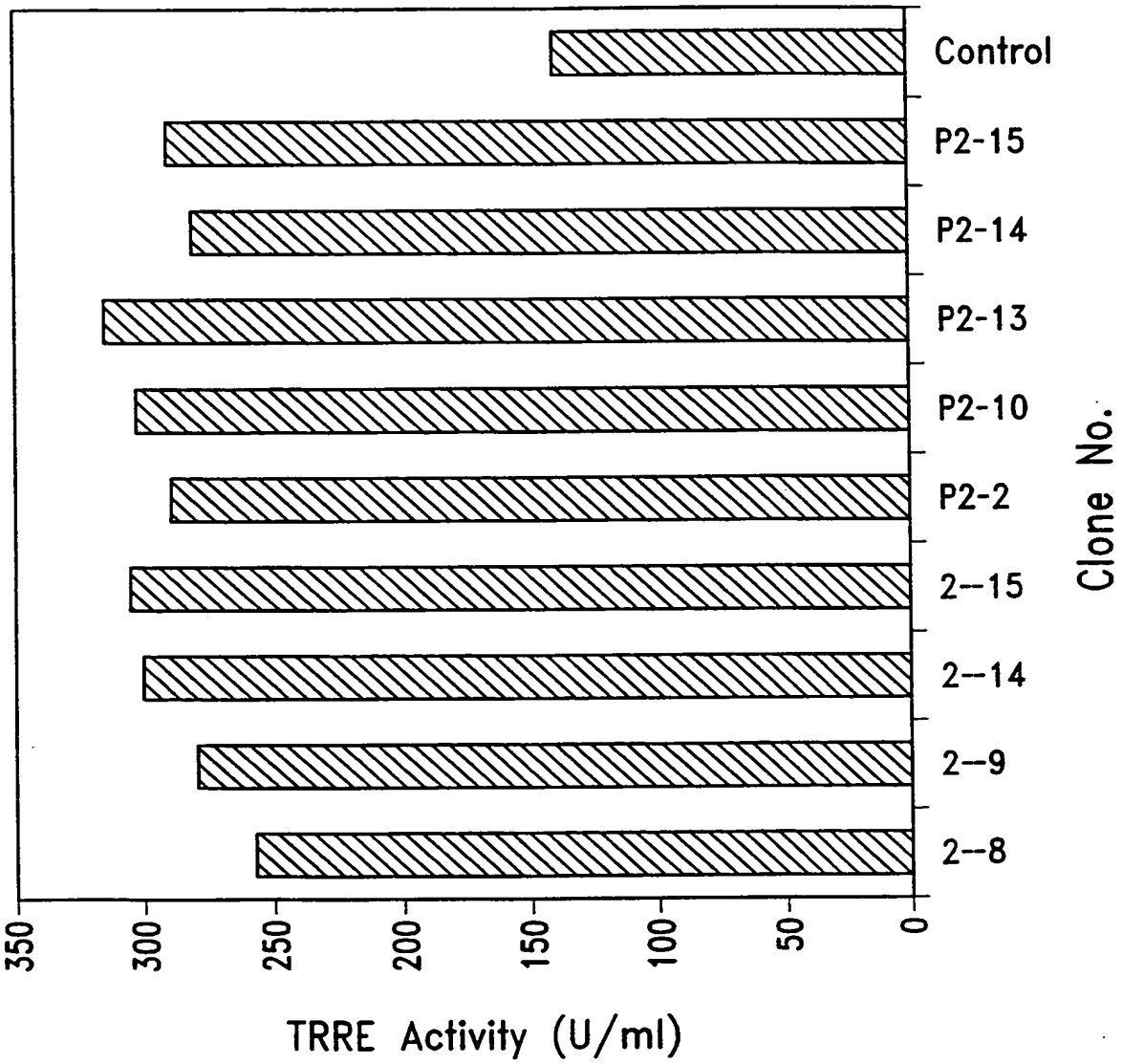
4
8
12
16
20
24
LPS only
LPS plus Control Buffer
LPS plus TRRE (2,000U)
LPS plus TRRE (4,000U)

Fig. 3

Fig. 4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2--8
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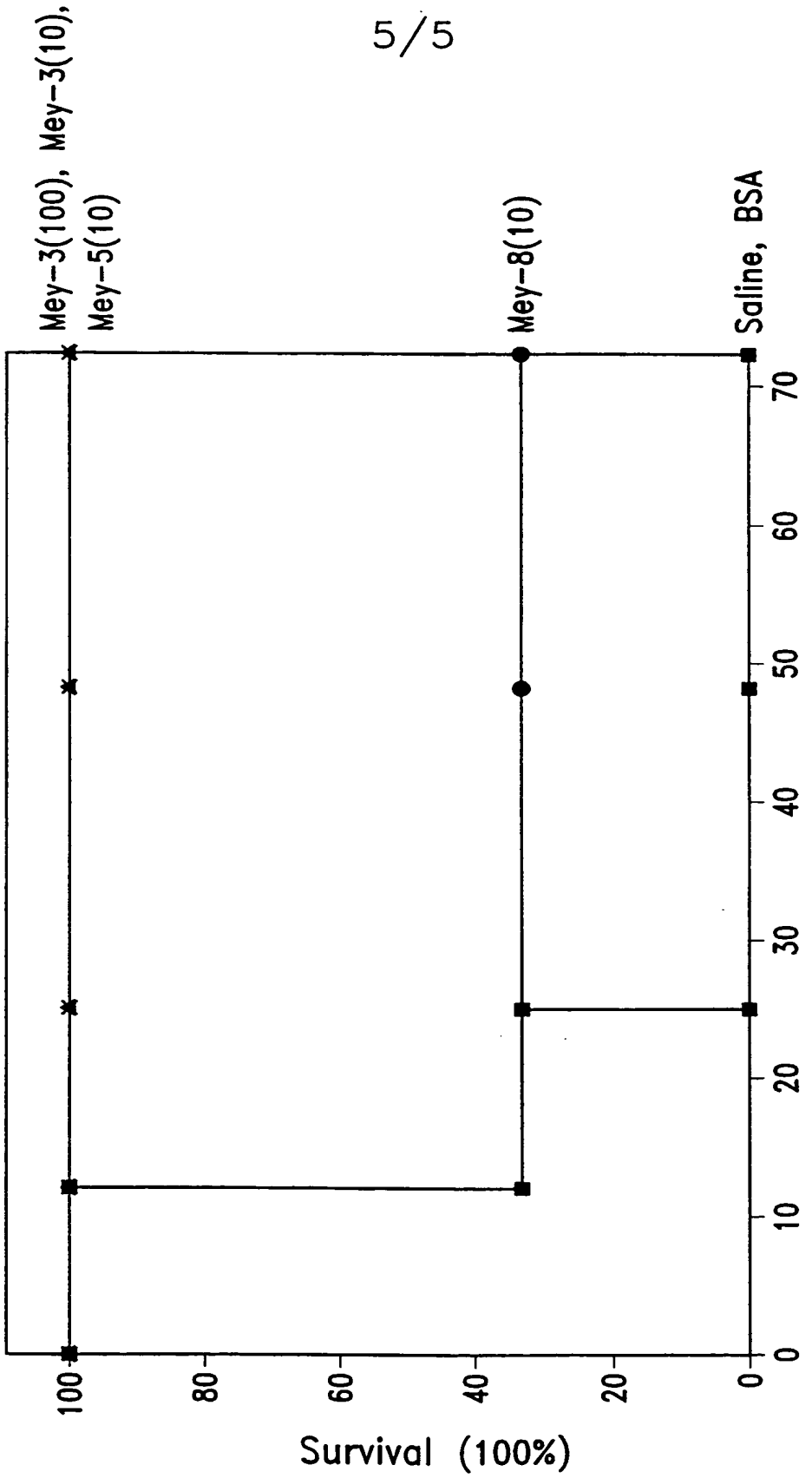
50
0
TRRE Activity (U/ml)


Mey-3(100), Mey-3(10),
Mey-5(10)
Mey-8(10)
Saline, BSA
Survival (100%)
100
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60
40
20
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Time post LPS (hr)
0
10
20
30
40
50
60
70

Fig. 5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Gatanaga, T.
Granger, G.A.

(ii) TITLE OF INVENTION: Factors Altering Tumor Necrosis Factor Receptor Releasing Enzyme Activity

(iii) NUMBER OF SEQUENCES: 154

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: MORRISON & FOERSTER
(B) STREET: 755 PAGE MILL ROAD
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304-1018

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows
(D) SOFTWARE: FastSEQ for Windows Version 2.0b

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: USSN 09/081,385
(B) FILING DATE: 014-NOV-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME:
(B) REGISTRATION NUMBER:
(C) REFERENCE/DOCKET NUMBER: 22000-20577.21

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-813-5600
(B) TELEFAX: 650-494-0792
(C) TELEX: 706141

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4047 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

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(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

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GGACTCCCCT GGGCCAATGA TGTTGCGTCT AATACCCTTT GTCTCTCCTC TATGCGTGCC  1980
CATTGCAGAG AAGGGGACTG GGACCAAAGG GGTGGGGATA ATGGGGAGCC CCATTGCTGG  2040
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CCTTGCATCT GAATAGGCCT ACCCTCACCA TTTATTCACT AATACATTTT ATTTGTGTTC  2100
TCTAATTTAA AATTACCTTT TCATCTTGCT TGATTTTCCT TCAGCTAAAT TAGAAATTTG  2160
TAGTTTTTCC CCTAAAAAAT TCAATGGCAT TCTTTCTTAT AAATTACATT CTCTGATTTT  2220
CTTGTCAGCC TGCTTCAAGG AAATCCATGT GTTCAAAATG CTTGCTCGCA GTTTGCTCCA  2280
TACCAAATGG TTGCTTAACC CAAATATCTG AGCAGCAAAT TGAGCTGATC CTTCTGGAGA  2340
AAGTACGGTT GAACAGCCAA GACCACTGGG TAGTCGAAGA GAAGACCACA CATCCTGAAC  2400
TCCCCAGTCT GGTGTGAGGG GAGGACAGCT GATAACTGGA TATGCAGTGT TCCCAGACAT  2460
CACTGGTCCC AAACCATTAC TTCTGCCTGC CACTGCCACA AATACAGTAG GAATGCCATC  2520
CCCTTCATAC TCAGCTTTAA TCCTCAGAGT TTCATCTGGT CCTTTATGCG CAGATGTTAC  2580
TCGAAGTTCA CATGGAATGC CAAAATTTCC ACAGGCCTTC TTGATTTTTT CACAGTGACC  2640
AAGATCAGAA GTAGAGCCCA TCAACACTAC AACCCTGCAC TGACTTTCTG ATTTCAAAAG  2700
CAACTCTACT CTCTCTGCAA CCCACTCAAA GTTTTTCTTT ACCATTTGGA GCCCTTCAGG  2760
AGTTACTTCT TTGAGGTCCC GATAAGACTG TTTGTCTTTC TGTTGGCTTC GATCTCCTGA  2820
TGGCCAGAGT CTCCAGGAAT CATTGTCAAT AACATCAGCA AGAACAATTT CTTTGGTGGT  2880
TACATCAACA CCAAATTCAA TCTTCATATC AACCAGTGTA CAATTCTGGG GCAACCAGGA  2940
TTTCTCCAGT ATTTCAAATA TAGCCTGTGT AGCATCTCGT GCCGAATTCA AAAAGCTT    2998
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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AAGCTTTTTG TGAAAACCCT AGGATATGTC CCCTCCCTCA CCACACCCAA CCCCCCGCCC    60
CTGCCCCAGG ACATGACGAT GCCTCACACA CACACACACA CACACATACA CACAAGGCCG   120
TGAGCTGCAC GCAGGAACAT GGGCTGCACT CACGACAACA TTGAAAAAAT ATACATTATA   180
TATGTACACC CGGGGCCCCC ACGTCCCCTC CCGTCCCCGC AGCCTGGCCA CACCAGGTCA   240
CGGAGGAGGG GCCGGGGCTG CAGGACCTCA GGACTGCAAG GGCAGGAAGG GAAACAGGAC   300
AAGAAAGGAA GGAAGTTGGA AAGGAGGGAG AAATGGGGTC CCCAGACTGA AATGGAAATG   360
AGGTGGGGCG ATCATAAGAG AAGCAGGGAC GATGGTCCAG CTGAGGGAGC CCTGCAGAGG   420
GGGAAAAGCT TCCCATGGAC AGGAGAGAGA AGGGAAGGGG AGAGGAGAGG GTTTCCTTCA   480
ATCCCACCCC CAGCCCCAGC CCCAGCCCCA GCCATTGCAA TCGTCACCCT CTCCCCAACA   540
CAGTGAGTGC TAAGGGGGCA GCTGCCATTG GGGGTAGAAA GGCAGCTGAA GTCCAGCCCA   600
CTTTCCAACC CAGCCAGCCC CAGTGCAAGG GGCACACCAG GAGCATGACA GCCCAGAAGT   660
GAGGGATGGG GGGCCGGGGG AGGGGCAGGG CGGACTCCAG AGGGCCCGCT GGGGTTTTGA   720
AATGAAAGGA GGACTGGTTC TGAAGCCTCT CTCCCTCTTG GTCTCTGTGT TCCCAGAAAG   780
TCCTTCTCCC ATGTCTGGAG TGTCTGTTTC ACCAGGGCAG AATTCCCCCT CTGCGTGGGG   840
AGAGGTGTAG GCCTTAGTAG CGGTGTGGGG GGGTCTCGAT GATGCGTCTC TCGTCGCTGC   900
TGGGGGAATC GGCCACCTCC GAGTCACTGC TGTCCTCATC CTCCTGCTGG CCCCCAACAG   960
CCCCCGTCAC ACAGGACTGC CGATTCTGGT AGGACTCCAT GGGGTTCACA ATGATGGTGA  1020
GAGCTGAGTC ATCCCAGAAG AGGTCTGGGT CCTTGGGGTC ACTGGAGGCC CCTGGAGGCC  1080
CGCCGGCCCC TGAGACGCGG CGGTGAAGGG AATGGATGCG CACCAGGCCC AGGACGACCA  1140
TGAGCACCAG GAAGCCCACG CACACCACAA TGATGAGGGT TGCGGCGCTG GGTATCATGG  1200
AGTTTCTGTG GGAGCTGGCT AGGCTGTGTC CAGCCATCTC AGGCGGGGGC TGGTGACCAC  1260
GGTGCAGGAA CTGCTGGGAG CTGAGCACGT GGCTGGGGTG GGCAACCCGG TTCATGCTGT  1320
GCAGGACATT GACCTCCACG ATGAATTCAT TGCTGGAGTA ACGGCCATTC ATTTCCGAGC  1380
AGGAAAGCCG GAACTTCCTG GTGTAGAGGG CAGCTCCGTG TCGCAGCCGA TAACGAGCCT  1440
GCCTCAGGAT CTCTTCATAC ACAGTGATGC TCTCCACCCC AGCAATAGTG AGGTAGGCAG  1500
ATGTGTTGGT GAGCTCCAGC CCCCGCTGCT GCAGAGAGGT TGTGTCCAGG AGCAGGCTTT  1560
CCCGCTCGGG ATCCAGGTCA TCCCCCACCA GAGAAATTTC ACAGCCATCC AGGTTGTGCA  1620
CAATCTCATC CGACATGCGT GTGTCTGTCA CTGTGCCCTG CCAACTCTCA TCCTTTTTGG  1680
CCTCCACCTG GTGAGAAATG GAGCAGGTGA TTTGAAGATC AGGGAACAAA GGGACGCCGT  1740
TGGTTCCCTC AAAGTCCACA GCTGGGCGGG CAAAATGAGC AGTGCCACTC AGCAGGATCT  1800
GGGGGGCGTC AGGCTGAAGG ACGACCACGT AGCCCTCCAC TTCAGGGATG GAGACGCAGG  1860
ACTCTTCGCT GAAGCACTTG ACAGCAGTGG TGAGGCGCAG GGGCCTGACG CCGGGCGTGG  1920
CAAAGCGCAG AGTGTTCATG TAAGCCACAT GCTGCAGGGC ATGGTTGAAG GTCTCCACAT  1980
CATCCCCCTC CAGGGTGAGC AGGGACTGTG AGGGGTTCAC GTGGACCTTC ATGCCTTTGC  2040
CCAGGCTCTC GAAATCCCTA TAGTCCAGCC CCTCCCGACA TGCATAGAGG CACTCGATGA  2100
CCTCGCGGCT CTCCAGGCGA CCTGAGCGCA CGCTGAAACC AGCCAGGTAG CCATGGAAGT  2160
AGTGGTGGAT CGACAAAGGG TCTCCTTGGG TGGTGTCTGT ACTGTTGTCT CCCTTTTCCT  2220
TCTCTTTGTT CTTCTCCTCA GTCCAGCAGG CCCCAATCAT GAGAGCAGGC TCCCTTCGGG  2280
GTGGGTGGAT GAGGCCATTG TCATGGATGA GGGCAGGGTC GAAGGAGATG CCGTCGGTAT  2340
AGAGTGTGAC TGTGGGGAAC TCGAGGTTCA GAGCGTAGTG GTGCCACTCA TCATCACAGA  2400
CCTGCTCCAG CTTCCAGAGG AACTTGACTG GGCGGGCACT CTCAAGCAGG GGCCAGTAGA  2460
GGAAGGCAAT CCTACAGCCG TGGACAGTCA GCGAGTAGTG AGAGAAGCCG TCCTCATTCT  2520
GGACAGTGTT ACATACGATG GTTTCCTCTT CCTTCTTGCC CTTGTTGGGA GTTACGCCAT  2580
GCTTCATCCA GAAGGACAGG GTGAAGTGGT CACTGAGGCT GTCCTGGGGC CCAGAGCCCA  2640
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GCCCACTGGG GCCACCCAGG GGCACCTGCA CAGCCTGGGT GCCATTGAAC CAGTAGATCA 2700
GGCTGCTGTC CTGGCTGTAG TGCACCGAGA GTCCTGCTGT CCAGTTGGCA TTGGGGCCAG 2760
GCATGGGCAA CAGATCCACT TCCCCAGTGG CAGCACCACA GAGTTTCCGC AGCGCCCGCT 2820
CTGAGTAGTT GTCACGGTCA CAGCCCTTGG CCACATGGCT GGTCTGCAGC TCTATGGTGG 2880
CCTGAATGTT CCAGAGTGGT TCATCACAGG TCTCCAGGCG GATACCAGGG AACAAAGCCA 2940
AGCTCCCAGC ACCTGGTGCA TATTCGATCC TTTTGTTCCA GCCTTGCCAG CTGGGTTTAC 3000
AGGTGGGCTT CACCTGAATC TCCACCTCAG CATCATCTGC TGCCCGCTTC TTCCCACAGT 3060
CATAAGCTGT CACTGTAAAC TTATAGAGCC TCTCACCACT GTACTGCAGC TTCTCTGTGT 3120
TCTCAATGTT CCCGTCATTG TCAATGAGGA AAGGGGTGTT GGGTGTGAGA ATCTCATAGT 3180
AGCAGATCTG GCTGTACTGG GGGGAGCAGT CACCGTCAAT GGCTTCCACC CGCAGGATGC 3240
GATCGTACAG CTTCCCCTCT GTCACAGCCG CACGATACAG CCGTTCCACA AACACTGGGG 3300
CAAACTCGTT CACATCGTTG ACCCGCACAT GCACAGTGGC CTTGTGGGAC TTCTTGGTGT 3360
TGGCCCCGTC GGGGCCCTCG CCACAGTCAT AGGCCTGGAT GGTGAAGGTG TGTTCCTTCT 3420
GGGCCTCGCA GTCCACAGGC TCCTTGGCCC GGATCAGCCC CTCTCCTGTC GCCTTGTCAA 3480
GGATCACAGC CTCAAAGGGC ACCCCAGACC CATGGAGCCG GAAGCCGCAG ATCTCACCTG 3540
CATAGCGCAG CGGGGCATCC TTGTCCAAGG CAAAGAGTGG TGGATTCAGT AGGACCGTGT 3600
TGTCATTCTC CATGACGATG CCCTGGTACT CTGCCTCAAT CCATGGCTTG TGCTTGTTGG 3660
CTTTGTTACA GGAGCAGGAC GCGAGCAGAG AGGCCAGCAG AAGGGGCAGC AGCAGGAGGG 3720
TCATGGTGCG GCGTGGGGCA GGGCAGGGCC AGGCGTTTGC CTCCCCTGGG AGCCTCCAGC 3780
CTGCGGATTC CACCTTGCGG GAGGGATACA GGGGGGGAAA ACCAAAATAA AACGTCAAAT 3840
AAATTGTGTA GGAGGAGTCC AGCTTAGGAC CGGGCCAGAG CCAGGCCAGG CTCGGGGAGG 3900
GGGCCTCTGC AGGTTCAGAG GATCACTGCT GCCACCACCG CCACCCTGGG AGCCAGTTAT 3960
TTTGCCATGG CCTTGATTGC AACAGCTGCC TCCTCTGTCA TGGCAGACAG CACCGTGATC 4020
AGGATCTCTT CTCCACAGTC GTACTTCTGC TCAATCTCCT TGCCAAGGTC TCCCTCAGGG 4080
AGACGAAGGT CCTCTCGTAC CTCCCCGCTG TCCTGGAGCA GTGATAGGTA CCCATCCTGG 4140
ATCTTTGGAT CC 4152

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGATCCAAAG ATTCGGCACG AGTGGCCACA TCATGAACCT CCAGGCCCAG CCCAAGGCTC 60
AGAACAAGCG GAAGCGTTGC CTCTTTGGGG GCCAGGAACC AGCTCCCAAG GAGCAGCCCC 120
CTCCCCTGCA GCCCCCCCAG CAGTCCATCA GAGTGAAGGA GGAGCAGTAC CTCGGGCACG 180
AGGGTCCAGG AGGGGCAGTC TCCACCTCTC AGCCTGTGGA ACTGCCCCCT CCTAGCAGCC 240
TGGCCCTGCT GAACTCTGTG GTGTATGGGC CTGAGCGGAC CTCAGCAGCC ATGCTGTCCC 300
AGCAGGTGGC CTCAGTAAAG TGGCCCAACT CTGTGATGGC TCCAGGGCGG GGCCCGGAGC 360
GTGGAGGAGG TGGGGGTGTC AGTGACAGCA GCTGGCAGCA GCAGCCAGGC CAGCCTCCAC 420
CCCATTCAAC ATGGAACTGC CACAGTCTGT CCCTCTACAG TGCAACCAAG GGGAGCCCGC 480
ATCCTGGAGT GGGAGTCCCG ACTTACTATA ACCACCCTGA GGCACTGAAG CGGGAGAAAG 540
CGGGGGGCCC ACAGCTGGAC CGCTATGTGC GACCAATGAT GCCACAGAAG GTGCAGCTGG 600
AGGTAGGGCG GCCCCAGGCA CCCCTGAATT CTTTCCACGC AGCCAAGAAA CCCCCAAACC 660
AGTCACTGCC CCTGCAACCC TTCCAGCTGG CATTCGGCCA CCAGGTGAAC CGGCAGGTCT 720
TCCGGCAGGG CCCACCGCCC CCAAACCCGG TGGCTGCCTT CCCTCCACAG AAGCAGCAGC 780
AGCAGCAGCA ACCACAGCAG CAGCAGCAGC AGCAGCAGGC AGCCCTACCC CAGATGCCGC 840
TCTTTGAGAA CTTCTATTCC ATGCCACAGC AACCCTCGCA GCAACCCCAG GACTTTGGCC 900
TGCAGCCAGC TGGGCCACTG GGACAGTCCC ACCTGGCTCA CCACAGCATG GCACCCTACC 960
CCTTCCCCCC CAACCCAGAT ATGAACCCAG AACTGCGCAA GGCCCTTCTG CAGGACTCAG 1020
CCCCGCAGCC AGCGCTACCT CAGGTCCAGA TCCCCTTCCC CCGCCGCTCC CGCCGCCTCT 1080
CTAAGGAGGG TATCCTGCCT CCCAGCGCCC TGGATGGGGC TGGCACCCAG CCTGGGCAGG 1140
AGGCCACTGG CAACCTGTTC CTACATCACT GGCCCCTGCA GCAGCCGCCA CCTGGCTCCC 1200
TGGGGCAGCC CCATCCTGAA GCTCTGGGAT TCCCGCTGGA GCTGAGGGAG TCGCAGCTAC 1260
TGCCTGATGG GGAGAGACTA GCACCCAATG GCCGGGAGCG AGAGGCTCCT GCCATGGGCA 1320
GCGAGGAGGG CATGAGGGCA GTGAGCACAG GGGACTGTGG GCAGGTGCTA CGGGGCGGAG 1380
TGATCCAGAG CACGCGACGG AGGCGCCGGG CATCCCAGGA GGCCAATTTG CTGACCCTGG 1440
CCCAGAAGGC TGTGGAGCTG GCCTCACTGC AGAATGCAAA GGATGGCAGT GGTTCTGAAG 1500
AGAAGCGGAA AAGTGTATTG GCCTCAACTA CCAAGTGTGG GGTGGAGTTT TCTGAGCCTT 1560
CCTTAGCCAC CAAGCGAGCA CGAGAAGACA GTGGGATGGT ACCCCTCATC ATCCCAGTGT 1620
CTGTGCCTGT GCGAACTGTG GACCCAACTG AGGCAGCCCA GGCTGGAGGT CTTGATGAGG 1680
ACGGGAAGGG TCTTGAACAG AACCCTGCTG AGCACAAGCC ATCAGTCATC GTCACCCGCA 1740
GGCGGTCCAC CCGAATCCCC GGGACAGATG CTCAAGCTCA GGCGGAGGAC ATGAATGTCA 1800
AGTTGGAGGG GGAGCCTTCC GTGCGGAAAC CAAAGCAGCG GCCCAGGCCC GAGCCCCTCA 1860
TCATCCCCAC CAAGGCGGGC ACTTTCATCG CCCCTCCCGT CTACTCCAAC ATCACCCCAT 1920
ACCAGAGCCA CCTGCGCTCT CCCGTGCGCC TAGCTGACCA CCCCTCTGAG CGGAGCTTTG 1980
AGCTACCTCC CTACACGCCG CCCCCCATCC TCAGCCCTGT GCGGGAAGGC TCTGGCCTCT 2040

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ACTTCAATGC CATCATATCA ACCAGCACCA TCCCTGCCCC TCCTCCCATC ACGCCTAAGA   2100
GTGCCCATCG CACGCTGCTC CGGACTAACA GTGCTGAAGT AACCCCGCCT GTCCTCTCTG   2160
TGATGGGGGA GGCCACCCCA GTGAGCATCG AGCCACGGAT CAACGTGGGC TCCCGGTTCC   2220
AGGCAGAAAT CCCCTTGATG AGGGACCGTG CCCTGGCAGC TGCAGATCCC CACAAGGCTG   2280
ACTTGGTGTG GCAGCCATGG GAGGACCTAG AGAGCAGCCG GGAGAAGCAG AGGCAAGTGG   2340
AAGACCTGCT GACAGCCGCC TGCTCCAGCA TTTTCCCTGG TGCTGGCACC AACCAGGAGC   2400
TGGCCCTGCA CTGTCTGCAC GAATCCAGAG GAGACATCCT GGAAACGCTG AATAAGCTGC   2460
TGCTGAAGAA GCCCCTGCGG CCCCACAACC ATCCGCTGGC AACTTATCAC TACACAGGCT   2520
CTGACCAGTG GAAGATGGCC GAGAGGAAGC TGTTCAACAA AGGCATTGCC ATCTACAAGA   2580
AGGATTTCTT CCTGGTGCAG AAGCTGATCC AGACCAAGAC CGTGGCCCAG TGCGTGGAGT   2640
TCTACTACAC CTACAAGAAG CAGGTGAAAA TCGGCCGCAA TGGGACTCTA ACCTTTGGGG   2700
ATGTGGATAC GAGCGATGAG AAGTCGGCCC AGGAAGAGGT TGAAGTGGAT ATTAAGACTT   2760
CCCAAAAGTT CCCAAGGGTG CCTCTTCCCA GAAGAGAGTC CCCAAGTGAA GAGAGGCTGG   2820
AGCCCAAGAG GGAGGTGAAG GAGCCCAGGA AGGAGGGGGA GGAGGAGGTG CCAGAGATCC   2880
AAGAGAAGGA GGAGCAGGAA GAGGGGCGAG AGCGCAGCAG GCGGGCAGCG GCAGTCAAAG   2940
CCACGCAGAC ACTACAGGCC AATGAGTCGG CCAGTGACAT CCTCATCCTC CGGAGCCACG   3000
AGTCCAACGC CCCTGGGTCT GCCGGTGGCC AGGCCTCGGA GAAGCCAAGG GAAGGGACAG   3060
GGAAGTCACG AAGGGCACTA CCTTTTTCAG AAAAAAAAAA AAAAAAACAA AAAGCTT      3117
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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3306 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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TTAATCCGCT AAACAGTTCG CAAGGATATA ACGTCTCTGG CATTGGGAAG ATTTTTATGT    180
TTAATGTCTG CGGCACAATG CCTGTCTGTG GGACCATCCT GGGAAAACCT GCTTCTGGCT    240
GTGAGGCAGA AACCCAAACT GAAGAGCTCA AGAATTGGAA GCCAGCAAGG CCAGTCGGAA    300
TTGAGAAAAG CCTCCAGCTG TCCACAGAGG GCTTCATCAC TCTGACCTAC AAAGGGCCTC    360
TCTCTGCCAA AGGTACCGCT GATGCTTTTA TCGTCCGCTT TGTTTGCAAT GATGATGTTT    420
ACTCAGGGCC CCTCAAATTC CTGCATCAAG ATATCGACTC TGGGCAAGGG ATCCGAAACA    480
CTTACTTTGA GTTTGAAACC GCGTTGGCCT GTGTTCCTTC TCCAGTGGAC TGCCAAGTCA    540
CCGACCTGGC TGGAAATGAG TACGACCTGA CTGGCCTAAG CACAGTCAGG AAACCTTGGA    600
CGGCTGTTGA CACCTCTGTC GATGGGAGAA AGAGGACTTT CTATTTGAGC GTTTGCAATC    660
CTCTCCCTTA CATTCCTGGA TGCCAGGGCA GCGCAGTGGG GTCTTGCTTA GTGTCAGAAG    720
GCAATAGCTG GAATCTGGGT GTGGTGCAGA TGAGTCCCCA AGCCGCGGCG AATGGATCTT    780
TGAGCATCAT GTATGTCAAC GGTGACAAGT GTGGGAACCA GCGCTTCTCC ACCAGGATCA    840
CGTTTGAGTG TGCTCAGATA TCGGGCTCAC CAGCATTTCA GCTTCAGGAT GGTTGTGAGT    900
ACGTGTTTAT CTGGAGAACT GTGGAAGCCT GTCCCGTTGT CAGAGTGGAA GGGGACAACT    960
GTGAGGTGAA AGACCCAAGG CATGGCAACT TGTATGACCT GAAGCCCCTG GGCCTCAACG   1020
ACACCATCGT GAGCGCTGGC GAATACACTT ATTACTTCCG GGTCTGTGGG AAGCTTTCCT   1080
CAGACGTCTG CCCCACAAGT GACAAGTCCA AGGTGGTCTC CTCATGTCAG GAAAAGCGGG   1140
AACCGCAGGG ATTTCACAAA GTGGCAGGTC TCCTGACTCA GAAGCTAACT TATGAAAATG   1200
GCTTGTTAAA AATGAACTTC ACGGGGGGGG ACACTTGCCA TAAGGTTTAT CAGCGCTCCA   1260
CAGCCATCTT CTTCTACTGT GACCGCGGCA CCCAGCGGCC AGTATTTCTA AAGGAGACTT   1320
CAGATTGTTC CTACTTGTTT GAGTGGCGAA CGCAGTATGC CTGCCCACCT TTCGATCTGA   1380
CTGAATGTTC ATTCAAAGAT GGGGCTGGCA ACTCCTTCGA CCTCTCGTCC CTGTCAAGGT   1440
ACAGTGACAA CTGGGAAGCC ATCACTGGGA CGGGGGACCC GGAGCACTAC CTCATCAATG   1500
TCTGCAAGTC TCTGGCCCCG CAGGCTGGCA CTGAGCCGTG CCCTCCAGAA GCAGCCGCGT   1560
GTCTGCTGGG TGGCTCCAAG CCCGTGAACC TCGGCAGGGT AAGGGACGGA CCTCAGTGGA   1620
GAGATGGCAT AATTGTCCTG AAATACGTTG ATGGCGACTT ATGTCCAGAT GGGATTCGGA   1680
AAAAGTCAAC CACCATCCGA TTCACCTGCA GCGAGAGCCA AGTGAACTCC AGGCCCATGT   1740
TCATCAGCGC CGTGGAGGAC TGTGAGTACA CCTTTGCCTG GCCCACAGCC ACAGCCTGTC   1800
CCATGAAGAG CAACGAGCAT GATGACTGCC AGGTCACCAA CCCAAGCACA GGACACCTGT   1860
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TGGTTTACAT GAGCATCTGT GGGGAGAATG AAAACTGCCC TCCTGGCGTG GGGGCCTGCT   1980
TTGGACAGAC CAGGATTAGC GTGGGCAAGG CCAACAAGAG GCTGAGATAC GTGGACCAGG   2040
TCCTGCAGCT GGTGTACAAG GATGGGTCCC CTTGTCCCTC CAAATCCGGC CTGAGCTATA   2100
AGAGTGTGAT CAGTTTCGTG TGCAGGCCTG AGGCCGGGCC AACCAATAGG CCCATGCTCA   2160
TCTCCCTGGA CAAGCAGACA TGCACTCTCT TCTTCTCCTG GCACACGCCG CTGGCCTGCG   2220
AGCAAGCGAC CGAATGTTCC GTGAGGAATG GAAGCTCTAT TGTTGACTTG TCTCCCCTTA   2280
TTCATCGCAC TGGTGGTTAT GAGGCTTATG ATGAGAGTGA GGATGATGCC TCCGATACCA   2340
ACCCTGATTT CTACATCAAT ATTTGTCAGC CACTAAATCC CATGCACGGA GTGCCCTGTC   2400
CTGCCGGAGC CGCTGTGTGC AAAGTTCCTA TTGATGGTCC CCCCATAGAT ATCGGCCGGG   2460
TAGCAGGACC ACCAATACTC AATCCAATAG CAAATGAGAT TTACTTGAAT TTTGAAAGCA   2520
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GTACTCCTTG CCAGGAATTC AGTTGTAAAT AAAATTGAAC CTGCTCAACA GCTGAGGGAG   2580
ACTAGAAATG ATGGGTCCAT ATCCTGGTGC ATTGTCATAC AATTCAAACA ATGGTGCAGC   2640
TACCAGCTTG TAATTTTTAG GGACTGCAAA CAAGGCTTTT TCTTGAAGCT GAACCAGAAA   2700
CAACTTCTTA TGTTCCTTAG GCTTTGTAAT ATGTGCAGGA ATATATGGAT ACTGAGGAGG   2760
TTCAAAATTT GGTCTCCACC AGTTACCAAT GCAATCGTCA ATGACCCAGT CTTGCAAAAC   2820
TCCATCCTGA CGACCCAGTA TCTCTGTCAT TAAGCGTTTT AGTCCTTCAA CTTCATCTTC   2880
TCCTGGGTTA AGTTCACCAC CAGGTAGTTT GAAGAAAGTT GTTCCCAGCT GCAGCAGTAA   2940
CACATGGGGT AGCCGGTGCT CATGTACAAT CAGAACCCCT TCTACAGTCC TCCTCATTCC   3000
AATTTTATCA AATTCTTCCC TCATGCGCTG AAATCTGGCT GCAACAGAGC TGTCCTTCTC   3060
GTAGAGGGGC TCTTTTGTAC CAAAAGTATA ATTGGTAAGA GGGTACAGGT TGATGGTGCG   3120
CTCCAGGGTG AGGGGCTTCG TCTGCTGGAT GTACTTGTTG CCGAACTGAG TGACCCCCCG   3180
GGGCCAGCCG GTCTGCGAGC GATTGGGCGG TACCACAGAC ATGCTGGCGA GCTCCGGCGC   3240
TGACGGCGAG CAGAAAGTGG CAGGCAGGGT AGACTTTCCC CGTGCGGGAA GCCTCGTGCC   3300
GAATTC                                                              3306
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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4218 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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TACACAGTGT ATGAGTCTCA CATGAAATAC CCGGATGTAA ATCCAAAGAA ACAGGAAGCA    120
GATTGGTGGT TGCCAGGGAC AAGGGCGGTG GGAGGAGAAA ATGGAGAGTA ACGGGACTTT    180
ACTTTTGGAG TGATGAGAAT GTTTTGGAGC TAGATAGAAG TGGTGGTTGT ACACCATTGT    240
GGATGTACTA CCACTTAATT GTTCACTTAA AAAGTTAATT TATGTGAATT GCATCTTAAT    300
TAAAAACAAG GATAACATTC CAACTCCTGG ACATTATCCT TCCTTTCCAT TTGATGTCAG    360
GCCCGTGTTA GAATTCTCAT CCGGTTTGGT CACTGCACTT AAGATGTGGA GAAATTAGGA    420
CGCACAGTTA AGAGGAAGGA TAACACTGAT TAAGGTAGTG CTTTTCTAGG TTTCCCCTAA    480
ACAATTTAAC AGATGGATAG TGGCACCACT TACGAGATGG AAAAACCAGC GGAAGGAAGA    540
TTTGGGGGAG AAGTTAAGTT TGTCTTGGGC CTGTGTTTTG CAACCTGAGT GTAAAAGACA    600
TATGTTAAGT CTTCAGTGGC GAAACACTAA AACTAGAAAT GGATCAGAAT TTTATCTTTG    660
GATGTGACTT CTCAAGGATG GTCTTGTCAC TTCAGTGCCT GGTCAAATGA CAAGATGGGC    720
AATCTTTTCC TGAAGGTCCA AGCACCTGAA CGTGGCAGGG TGACCCGATT CCGATTTGCT    780
TAGAACAATC CTAGTTCATG CCTATTGTCC CTCATGTAAT TAATATCACT CTCAAAATGT    840
CTCATTTTGT GCAATAAATT CTGCAACGTG ATGGCGCGAC TCTCGCGGCC CGAGCGGCCG    900
GACCTTGTCT TCGAGGAAGA GGACCTCCCC TATGAGGAGG AAATCATGCG GAACCAATTC    960
TCTGTCAAAT GCTGGCTTCA CTACATCGAG TTCAAACAGG GCGCCCCGAA GCCCAGGCTC   1020
AATCAGCTAT ACGAGCGGGC ACTCAAGCTG CTGCCCTGCA GCTACAAACT CTGGTACCGA   1080
TACCTGAAGG CGCGTCGGGC ACAGGTGAAG CATCGCTGTG TGACCGACCC TGCCTATGAA   1140
GATGTCAACA ACTGTCATGA GAGGGCCTTT GTGTTCATGC ACAAGATGCC TCGTCTGTGG   1200
CTAGATTACT GCCAGTTCCT CATGGACCAG GGGCGCGTCA CACACACCCG CCGCACCTTC   1260
GACCGTGCCC TCCGGGCACT GCCCATCACG CAGCACTCTC GAATTTGGCC CCTGTATCTG   1320
CGCTTCCTGC GCTCACACCC ACTGCCTGAG ACAGCTGTGC GAGGCTATCG GCGCTTCCTC   1380
AAGCTGAGTC CTGAGAGTGC AGAGGAGTAC ATTGAGTACC TCAAGTCAAG TGACCGGCTG   1440
GATGAGGCCG CCCAGCGCCT GGCCACCGTG GTGAACGACG AGCGTTTCGT GTCTAAGGCC   1500
GGCAAGTCCA ACTACCAGCT GTGGCACGAG CTGTGCGACC TCATCTCCCA GAATCCGGAC   1560
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CAGCTGGGCA AGCTCTGGTG TTCTCTCGCC GACTACTACA TCCGCAGCGG CCATTTCGAG   1680
AAGGCTCGGG ACGTGTACGA GGAGGCCATC CGGACAGTGA TGACCGTGCG GGACTTCACA   1740
CAGGTGTTTG ACAGCTACGC CCAGTTCGAG GAGAGCATGA TCGCTGCAAA GATGGAGACC   1800
GCCTCGGAGC TGGGGCGCGA GGAGGAGGAT GATGTGGACC TGGAGCTGCG CCTGGCCCGC   1860
TTCGAGCAGC TCATCAGCCG GCGGCCCCTG CTCCTCAACA GCGTCTTGCT GCGCCAAAAC   1920
CCACACCACG TGCACGAGTG GCACAAGCGT GTCGCCCTGC ACCAGGGCCG CCCCCGGGAG   1980
ATCATCAACA CCTACACAGA GGCTGTGCAG ACGGTGGACC CCTTCAAGGC CACAGGCAAG   2040
CCCCACACTC TGTGGGTGGC GTTTGCCAAG TTTTATGAGG ACAACGGACA GCTGGACGAT   2100
GCCCGTGTCA TCCTGGAGAA GGCCACCAAG GTGAACTTCA AGCAGGTGGA TGACCTGGCA   2160
AGCGTGTGGT GTCAGTGCGG AGAGCTGGAG CTCCGACACG AGAACTACGA TGAGGCCTTG   2220
CGGCTGCTGC GAAAGGCCAC GGCGCTGCCT GCCCGCCGGG CCGAGTACTT TGATGGTTCA   2280
GAGCCCGTGC AGAACCGCGT GTACAAGTCA CTGAAGGTCT GGTCCATGCT CGCCGACCTG   2340
GAGGAGAGCC TCGGCACCTT CCAGTCCACC AAGGCCGTGT ACGACCGCAT CCTGGACCTG   2400
CGTATCGCAA CACCCCAGAT CGTCATCAAC TATGCCATGT TCCTGGAGGA GCACAAGTAC   2460
TTCGAGGAGA GCTTCAAGGC GTACGAGCGC GGCATCTCGC TGTTCAAGTG GCCCAACGTG   2520
TCCGACATCT GGAGCACCTA CCTGACCAAA TTCATTGCCC GCTATGGGGG CCGCAAGCTG   2580
GAGCGGGCAC GGGACCTGTT TGAACAGGCT CTGGACGGCT GCCCCCCAAA ATATGCCAAG   2640
ACCTTGTACC TGCTGTACGC ACAGCTGGAG GAGGAGTGGG GCCTGGCCCG GCATGCCATG   2700
GCCGTGTACG AGCGTGCCAC CAGGGCCGTG GAGCCCGCCC AGCAGTATGA CATGTTCAAC   2760
```

```
ATCTACATCA AGCGGGCGGC CGAGATCTAT GGGGTCACCC ACACCCGCGG CATCTACCAG   2820
AAGGCCATTG AGGTGCTGTC GGACGAGCAC GCGCGTGAGA TGTGCCTGCG GTTTGCAGAC   2880
ATGGAGTGCA AGCTCGGGGA GATTGACCGC GCCCGGGCCA TCTACAGCTT CTGCTCCCAG   2940
ATCTGTGACC CCCGGACGAC CGGCGCGTTC TGGCAGACGT GGAAGGACTT TGAGGTCCGG   3000
CATGGCAATG AGGACACCAT CAAGGAAATG CTGCGTATCC GGCGCAGCGT GCAGGCCACG   3060
TACAACACGC AGGTCAACTT CATGGCCTCG CAGATGCTCA AGGTCTCGGG CAGTGCCACG   3120
GGCACCGTGT CTGACCTGGC CCCTGGGCAG AGTGGCATGG ACGACATGAA GCTGCTGGAA   3180
CAGCGGGCAG AGCAGCTGGC GGCTGAGGCG GAGCGTGACC AGCCCTTGCG CGCCCAGAGC   3240
AAGATCCTGT TCGTGAGGAG TGACGCCTCC CGGGAGGAGC TGGCAGAGCT GGCACAGCAG   3300
GTCAACCCCG AGGAGATCCA GCTGGGCGAG GACGAGGACG AGGACGAGAT GGACCTGGAG   3360
CCCAACGAGG TTCGGCTGGA GCAGCAGAGC GTGCCAGCCG CAGTGTTTGG GAGCCTGAAG   3420
GAAGACTGAC CCGTCCCCTC GTGCCGAATT CGGCACGAGC AAGACCAGCC CCCAGATCAT   3480
TTGCCTCAAA GGTTTTCCCT CGAAGTCACA AATGTTTCAA GGAATCTCAA ATTTTACAAA   3540
GTTTGAAGTG TGGGCATTGG TGGCCTGTGG CTGTGTCCTC TCTCTGTAGC TGTTTTCTCC   3600
CTACATCCCT GAAAGGAAGT TGAGCCTGCT CCTCCATCCG CAGACCTCCC TTTCCAGCGC   3660
CCAGGGCATG GGGTGCTGTG AGGGCAGCAT GCTAGGTGTG ACCGTGCTCC TGGCCTCCAG   3720
GCCCGTGTCC CTCTGTCCTC TAGCCCACTA AGGCCCTGGC CCATTTGTGC TAAACAGGCA   3780
GTCGGACCTA GAAAGAGCAG ACAATCTCTC TGGGTCACCA GTCTGGCTAG GAGCTGGTCT   3840
CCTGACTGGG ATCCAGGCCT TCTCCCCTGC CCATGTGAAT TCCCAGGGGC AGAGCCTGAA   3900
ATGTTGAACA CAGCACTGGC CAAAGAGATG TCACCGTGGG AACCGAGGCT CTCTTCTCCT   3960
CCTGCCTGCT TTCGTGGGTT CAGAGTAGCT GAGGCTTGTC TGAGAGGAGT TGGAGTGCTG   4020
GTTTTCACCC TGGTTGGTGT GCTTTGCTTT GAGGGCACTT AGAAAGCCCA GCCCAGCCCT   4080
TGCTCCTGCC CTGCACACAG CGGAGCGACT TTTCTAGGTA TGCTCTTGAT TTCTGCAGAA   4140
GCAGCAGGTG GCATGGAGCC AAGAGGAAGT GTGACTGAAA CTGTCCACTC ATAGCCCGGC   4200
TGCCGTATTG AGAGGGCT                                                 4218
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1187 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GAGCTCGCGC GCCTGCAGGT CGACACTAGT GGATCCAAAG AATTCGGCAC GAGGGAAACT     60
CAACGGTGTA CGAGTGGAGG ACAGGGACAG AGCCCTCTGT GGTGGAACGA CCCCACCTCG    120
AGGAGCTTCC TGAGCAGGTG GCAGAAGATG CGATTGACTG GGGCGACTTT GGGGTAGAGG    180
CAGTGTCTGA GGGGACTGAC TCTGGCATCT CTGCCGAGGC TGCTGGAATC GACTGGGGCA    240
TCTTCCCGGA ATCAGATTCA AAGGATCCTG GAGGTGATGG GATAGACTGG GGAGACGATG    300
CTGTTGCTTT GCAGATCACA GTGCTGGAAG CAGGAACCCA GGCTCCAGAA GGTGTTGCCA    360
GGGGCCCAGA TGCCCTGACA CTGCTTGAAT ACACTGAGAC CCGGAATCAG TTCCTTGATG    420
AGCTCATGGA GCTTGAGATC TTCTTAGCCC AGAGAGCAGT GGAGTTGAGT GAGGAGGCAG    480
ATGTCCTGTC TGTGAGCCAG TTCCAGCTGG CTCCAGCCAT CCTGCAGGGC CAGACCAAAG    540
AGAAGATGGT TACCATGGTG TCAGTGCTGG AGGATCTGAT TGGCAAGCTT ACCAGTCTTC    600
AGCTGCAACA CCTGTTTATG ATCCTGGCCT CACCAAGGTA TGTGGACCGA GTGACTGAAT    660
TCCTCCAGCA AAAGCTGAAG CAGTCCCAGC TGCTGGCTTT GAAGAAAGAG CTGATGGTGC    720
AGAAGCAGCA GGAGGCACTT GAGGAGCAGG CGGCTCTGGA GCCTAAGCTG GACCTGCTAC    780
TGGAGAAGAC CAAGGAGCTG CAGAAGCTGA TTGAAGCTGA CATCTCCAAG AGGTACAGCG    840
GGCGCCCTGT GAACCTGATG GGAACCTCTC TGTGACACCC TCCGTGTTCT TGCCTGCCCA    900
TCTTCTCCGC TTTTGGGATG AAGATGATAG CCAGGGCTGT TGTTTTGGGG CCCTTCAAGG    960
CAAAAGACCA GGCTGACTGG AAGATGGAAA GCCACAGGAA GGAAGCGGCA CCTGATGGTG   1020
ATCTTGGCAC TCTCCATGTT CTCTACAAGA AGCTGTGGTG ATTGGCCCTG TGGTCTATCA   1080
GGCGAAAACC ACAGATTCTC CTTCTAGTTA GTATAGCGCA AAAAGCTTCT CGAGAGTACT   1140
TCTAGAGCGG CCGCGGGCCC ATCGATTTTC CACCCGGGTG GGGTACC                  1187
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3306 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
CCCTCACTAA AGGGAACAAA AGCTGGAGCT CGCGCGCCTG CAGGTCGACA CTAGTGGATC     60
GAAAGTTCGT TACGCCAAGC TCGAAATTAA CTCTGGGCTG ACCCATAAAC ATTTGTCTGA    120
```

```
TCTAGGATAT AGTTGCGTTT CTTGCGGGCA GCAATCTGGA TGAGGCGGTT GAGGCACTGG    180
GTGGCCTGCT GGATCAGGAC ATCCCAGCGG CCAGCATAGT TCCGCTGCCG GCGTAGGCCC    240
ATCACCCGCA TCTTATCCAT GATGGCATTG GTACCCAGGA TGTTGTACTT CTTGGAAGGG    300
TTGGAGGCTG CATGTTTGAT GGCCCATGTG GTCTTGCCAG CAGCAGGCAG GCCCACCATC    360
ATCAGAATCT CACATTCTGC CTTGCTCTTT GGTCCAACGG TGCCCCGGAT ACGCTCACTA    420
AGGGGAAGGT GCTGGATGAA GGTAAACCCC GGGAGGACAG AACAGTAGGG CTCTGCTCTC    480
TGTCCGAAGT TGAACTCCAC TGCGCAATTC TTCACCAGGA CATGAGGATA GAGGGCCTGA    540
CCCCCCAAGG CTTCCTTCTG GATTCGGAAA GCAATGCCCA TCCACTTTCC ATTCTTGGTA    600
AAAGACAGTT CCACGTCATT TCCACATTCA AAATCCGCAA AGCAGCCAAT CACCGGAGAG    660
CTCTGCGGTG CTAGGAGAGC GGCTGGGCCC GCAGACTGGG GGGAAAGCTC CGCAGCCGCA    720
GTGGGCCCCA GGATCAGGCC CCGCGTGGCC TGGAGAAGCC CAGTCTGGGC TGGAGCGGGA    780
GCTGGACAGT GTGGCCTTGC GTTCGCCCCC GGGAGCGCTG CGAGTGTCGC GGCCTCGGGT    840
GGATTTGCTG AGCACCAATA CCTCACGGTT GCCAACCTGG GGTTTTAGCT CCCTTGGTTT    900
TAATCCCCTA GGGGCGGGTG GGGGCACGGG AGGAAGGATG GGCCAGCTGG GTGCAATCCT    960
GCTGTAAGCC AGCCATTCCT TGATTTCTTA GAATTAACTA AACGGTCGCG CCGGAGGCCG   1020
CGGGGGCCGG AGCGGAGCAG CCGCGGCTGA GGTTCCCGAG TCGGCCGCTC GGGGCTGCGC   1080
TCCGCCGCCG GGACCCCGGC CTCTGGCCGC GCCGGCTCCG GCCTCCGGGG GGGCCGGGGC   1140
CGCCGGGACA TGGTGCCAGT CGCACCCCTT CCCCGCCGCC GCTGAGCTCG CCGGCCGCGC   1200
CCGGGCTGGG ACGTCCGAGC GGGAAGATGT TTTCCGCCCT GAAGAAGCTG GTGGGGTCGG   1260
ACCAGGCCCC GGGCCGGGAC AAGAACATCC CCGCCGGGCT GCAGTCCATG AACCAGGCGT   1320
TGCAGAGGCG CTTCGCCAAG GGGGTGCAGT ACAACATGAA GATAGTGATC CGGGGAGACA   1380
GGAACACGGG CAAGACAGCG CTGTGGCACC GCCTGCAGGG CCGGCCGTTC GTGGAGGAGT   1440
ACATCCCCAC ACAGGAGATC CAGGTCACCA GCATCCACTG GAGCTACAAG ACCACGGATG   1500
ACATCGTGAA GGTTGAAGTC TGGGATGTAG TAGACAAAGG AAAATGCAAA AAGCGAGGCG   1560
ACGGCTTAAA GATGGAGAAC GACCCCCAGG AGNCGGAGTC TGAAATGGCC CTGGATGCTG   1620
AGTTCCTGGA CGTGTACAAG AACTGCAACG GGGTGGTCAT GATGTTCGAC ATTACCAAGC   1680
AGTGGACCTT CAATTACATT CTCCGGGAGC TTCCAAAAGT GCCCACCCAC GTGCCAGTGT   1740
GCGTGCTGGG GAACTACCGG GACATGGGCG AGCACCGAGT CATCCTGCCG GACGACGTGC   1800
GTGACTTCAT CGACAACCTG GACAGACCTC CAGGTTCCTC CTACTTCCGC TATGCTGAGT   1860
CTTCCATGAA GAACAGCTTC GGCCTAAAGT ACCTTCATAA GTTCTTCAAT ATCCCATTTT   1920
TGCAGCTTCA GAGGGAGACG CTGTTGCGGC AGCTGGAGAC GAACCAGCTG GACATGGACG   1980
CCACGCTGGA GGAGCTGTCG GTGCAGCAGG AGACGGAGGA CCAGAACTAC GGCATCTTCC   2040
TGGAGATGAT GGAGGCTCGC AGCCGTGGCC ATGCGTCCCC ACTGGCGGCC AACGGGCAGA   2100
GCCCATCCCC GGGCTCCCAG TCACCAGTCC TGCCTGCACC CGCTGTGTCC ACGGGGAGCT   2160
CCAGCCCCGG CACACCCCAG CCCGCCCCAC AGCTGCCCCT CAATGCTGCC CCACCATCCT   2220
CTGTGCCCCC TGTACCACCC TCAGAGGCCC TGCCCCCACC TGCGTGCCCC TCAGCCCCCG   2280
CCCCACGGCG CAGCATCATC TCTAGGCTGT TTGGGACGTC ACCTGCCACC GAGGCAGCCC   2340
CTCCACCTCC AGAGCCAGTC CCGGCCGCAC AGGGCCCAGC AACGGTCCAG AGTGTGGAGG   2400
ACTTTGTTCC TGACGACCGC CTGGACCGCA GCTTCCTGGA AGACACAACC CCCGCCAGGG   2460
ACGAGAAGAA GGTGGGGGCC AAGGCTGCCC AGCAGGACAG TGACAGTGAT GGGGAGGCCC   2520
TGGGCGGCAA CCCGATGGTG GCAGGGTTCC AGGACGATGT GGACCTCGAA GACCAGCCAC   2580
GTGGGAGTCC CCCGCTGCCT GCAGGCCCCG TCCCCAGTCA AGACATCACT CTTTCGAGTG   2640
AGGAGGAAGC AGAAGTGGCA GCTCCCACAA AAGGCCCTGC CCCAGCTCCC CAGCAGTGCT   2700
CAGAGCCAGA GACCAAGTGG TCCTCCATAC CAGCTTCGAA GCCACGGAGG GGGACAGCTC   2760
CCACGAGGAC CGCAGCACCC CCCTGGCCAG GCGGTGTCTC TGTTCGCACA GGTCCGGAGA   2820
AGCGCAGCAG CACCAGGCCC CCTGCTGAGA TGGAGCCGGG GAAGGGTGAG CAGGCCTCCT   2880
CGTCGGAGAG TGACCCCGAG GGACCCATTG CTGCACAAAT GCTGTCCTTC GTCATGGATG   2940
ACCCCGACTT TGAGAGCGAG GGATCAGACA CACAGCGCAG GGCGGATGAC TTTCCCGTGC   3000
GAGATGACCC CTCCGATGTG ACTGACGAGG ATGAGGGCCC TGCCGAGCCG CCCCCACCCC   3060
CCAAGCTCCC TCTCCCCGCC TTCAGACTGA AGAATGACTC GGACCTCTTC GGGCTGGGGC   3120
TGGAGGAGGC CGGACCCAAG GAGAGCAGTG AGGAAGGTAA GGAGGGCAAA ACCCCCTCTA   3180
AGGAGAAGAA AAAAAAAACA AAAAGCTTCT CGAGAGTACT TCTAGAGCGG CCGCGGGCCC   3240
ATCGATTTTC CACCCGGGTG GGGTACCAGG TAAGTGTACC CAATTCGCCC TATAGTGAGT   3300
CGTATT                                                              3306
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TGCGGGGCCA GAGTGGGCTG                                               20
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCAGTCCTGG CCTGCGGATG 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCGACAGGA GAATTGGTTC 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCTGGGTTC GGTGCGGGAC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGGTCGGGTG TTTGTGAGTG 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCTCTTCCGT CTCCTCAGTG 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATTGCTAG TCTCACAGAC 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTAAGGGTGG CTGAAGGGAC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACCTTCCCTC CCTGTCACAG 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGGTCGGGTG TTTGTGAGTG 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ACACCATTCC AGAAATTCAG 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AAACTGCAGG TGGCTGAGTC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTCCTAATGT TTTCAGGGAG 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAACCTATG GTTACAATTC 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCCTAGACAT GGTTCAAGTG 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATATAATTA GTTCTCCATC 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGCCTGTTC CAGGCTGCAC 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGACGGCGAC CTCCACCCAC 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGGCTCCTCC GACGCCTGAG 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGTCTAGCCC TGGCCTTGAC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTCACTGGGG ACTCCGGCAG 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGCTTTCCC TGGGCACATG 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CACAGCTGTC TCAAGCCCAG 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACTGTTCCCC CTACATGATG 20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATCATATCCT CTTGCTGGTC 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTTCCCAGAG CTTGTCTGTG 20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTTGGCAGA CTCATAGTTG 20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TAGCAGGGAG CCATGACCTG 20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTTGGCGCCA GAAGCGAGAG 20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCTCTCTCTC TCTCTCTCTC 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCCCCGCTGA TTCCGCCAAG 20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTTTTGAAT TCGGCACGAG 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CCCCTGGTCC GCACCAGTTC 20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GAGAAGGGTC GGGGCGGCAG 20

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AAATCACATC GCGTCAACAC 20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TAAGAGAGTC ATAGTTACTC 20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCTCTAGAAG TACTCTCGAG 20

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACTCTGGCCA TCAGGAGATC 20

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CAGGCGTTGT AGATGTTCTG 20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AGTGGCAGGC AGAAGTAATG 20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGTTGGAGAA CTGGATGTAG 20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CTATTCAGAT GCAACGCCAG 20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CCATGGCACA CAGAGCAGAC 20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GCTACCATGC AGAGACACAG 20

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CAGGCTGACA AGAAAATCAG 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGCACGCATA GAGGAGAGAC 20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TGGGTGATGC CTTTGCTGAC 20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AAAACAAGAT CAAGGTGATG 20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TTGCCCACAT TGCTATGGTG 20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GACCAAGATC AGAAGTAGAG 20

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CCCCTGGGCC AATGATGTTG 20

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TCTTCCCACC ATAGCAATG 19

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TGGTCTTGGT GACCAATGTG 20

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ACACCTCGGT GACCCCTGTG 20

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TCTCCAAGTT CGGCACAGTG 20

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ACATGGGCTG CACTCACGAC 20

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GATCCTCTGA ACCTGCAGAG 20

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GGAAATGAGG TGGGGCGATC 20

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTTTGCCTTG GACAAGGATG 20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GCACCTGCCA TTGGGGGTAG 20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGTGGAAGCC ATTGACGGTG 20

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

TGCGTCTCTC GTCGCTGCTG 20

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GCGGAAACTC TGTGGTGCTG 20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

AGGATTGCCT TCCTCTACTG 20

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TGTCTGTTTC ACCAGGGCAG 20

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CCAGTGCCTC TATGCATGTC 20

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

AGGAAGCCCA CGCACACCAC 20

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CCCTTTGTTC CCTGATCTTC 20

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CGCTCGGGAT CCAGGTCATC 20

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TCGAGGTTCA GAGCGTAGTG 20

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TCTTGGATCT CTGGCACCTC 20

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CCATCAGAGT GAAGGAGGAG 20

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CCATCTTCCA CTGGTCAGAG 20

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CTCCTTCTCT TGGATCTCTG 20

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TTACTTCAGC ACTGTTAGTC 20

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

AGGGAGGTAG CTCAAAGCTC 20

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TGGGTCCACA GTTCGCACAG 20

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CAACTCTGTG ATGGCTCCAG 20

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

AGCAGGGTTC TGTTCAAGAC 20

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CCATTGGGTG CTAGTCTCTC 20

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CAGCCATGCT GTCCCAGCAG 20

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CTGGACCTGA GGTAGCGCTG 20

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ATAACCACCC TGAGGCACTG 20

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CCTGCAGGTC GACACTAGTG 20

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

AATTGGAATG AGGAGGACTG 20

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GCTCTAGAAG TACTCTCGAG 20

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

ATTGTATGAC AATGCACCAG 20

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TCCACAGAGG GCTTCATCAC 20

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CCTGACTGGC CTAAGCACAG 20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

AAGCCTCATA ACCACCAGTG 20

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

TGTCAACGGT GACAAGTGTG 20

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

TTGTACACCA GCTGCAGGTC 20

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GGGTGTGGTG CAGATGAGTC 20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

ATCACACTCT TATAGCTCAG 20

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GTGGGAAGCT TTCCTCAGAC 20

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TGATGAACAT GGGCCTGGAG 20

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

CATTGTGGAT GTACTACCAC 20

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

TGTGTTTTGC AACCTGAGTG 20

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

ATAGTGGCAC CACTTACGAG 20

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

AATTCTGCAA CGTGATGGCG 20

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

CACAAGATGC CTCGTCTGTG 20

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

AATCCGGACA AGGTACAGTC 20

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

GCACGAGTGG CACAAGCGTG 20

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

GCAAGCGTGT GGTGTCAGTG 20

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

TGTTTGAACA GGCTCTGGAC 20

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

CGGCATGGCA ATGAGGACAC 20

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

AGGACGAGAT GGACCTCCAG 20

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

CCCTCTGTCC TCTAGCCCAC 20

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TCTTGAGGGG ACTGACTCTG 20

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

TGAGTGAGGA GGCAGATGTC 20

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

TGGCTTTGAA GAAAGAGCTG 20

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GCAAAAGACC AGGCTGACTG 20

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

TGCAGCTCCT TGGTCTTCTC 20

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GATTCACAGT CCCAAGGCTC 20

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

ATCTGGATGA GGCGGTTGAG 20

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

GGTCACTCTC CGACGAGGAG 20

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

GGATCCAAAG TTCGTCTCTG 20

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

CGCTGTGTGT CTGATCCCTC 20

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

ATGAAGGTAA ACCCCGGGAG 20

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

TGGTCTCTGG CTCTGAGCAC 20

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

GCCTGGAGAA GCCCAGTCTG 20

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

CACACTCTGG ACCGTTGCTG 20

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

AAAGCTCCGC AGCCGCAGTG 20

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

TCTTCCAGGA AGCTGCGGTC 20

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

GATGGTGGGG CAGCATTGAG 20

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

GTCACCAGTG GTGCCTGCAG 20

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

ACCTCACGGT TGCCAACCTG 20

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

CGCAACAGCG TCTCCCTCTG 20

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

AGTACCTTCA TAAGTTCTTC 20

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

TCCCAGACTT CAACCTTCAC 20

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

AAACATCTTC CCGGTCGGAC 20

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GCTGAGCACC TTTACCTCAC 20

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GACGTCCGTC CGGGAAGATG 20

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

ACACAGGAGA TGCAGGTCAC 20

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GAGTCTTCCA TGAAGAACAG 20

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

GCAGTGAGGA AGGTAAGGAG 20

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4047 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 378...1799
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

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GGATCCAAAG GACGCCCCCG CCGACAGGAG AATTGGTTCC CGGGCCCGCG GCGATGCCCC      60
CCCGGTAGCT CGGGCCCGTG GTCGGGTGTT TGTGAGTGTT TCTATGTGGG AGAAGGAGGA     120
GGAGGAGGAA GAAGAAGCAA CGATTTGTCT TCTCGGCTGG TCTCCCCCCG GCTCTACATG     180
TTCCCCGCAC TGAGGAGACG GAAGAGGAGC CGTAGCCGCC CCCCCTCCCG GCCCGGATTA     240
TAGTCTCTCG CCACAGCGGC CTCGGCCTCC CCTTGGATTC AGACGCCGAT TCGCCCAGTG     300
TTTGGGAAAT GGGAAGTAAT GACAGCTGGC ACCTGAACTA AGTACTTTTA TAGGCAACAC     360
CATTCCAGAA ATTCAGG ATG AAT GGG GAT ATG CCC CAT GTC CCC ATT ACT         410
                  Met Asn Gly Asp Met Pro His Val Pro Ile Thr
                   1               5                  10

ACT CTT GCG GGG ATT GCT AGT CTC ACA GAC CTC CTG AAC CAG CTG CCT       458
Thr Leu Ala Gly Ile Ala Ser Leu Thr Asp Leu Leu Asn Gln Leu Pro
            15                  20                  25

CTT CCA TCT CCT TTA CCT GCT ACA ACT ACA AAG AGC CTT CTC TTT AAT       506
Leu Pro Ser Pro Leu Pro Ala Thr Thr Thr Lys Ser Leu Leu Phe Asn
        30                  35                  40

GCA CGA ATA GCA GAA GAG GTG AAC TGC CTT TTG GCT TGT AGG GAT GAC       554
Ala Arg Ile Ala Glu Glu Val Asn Cys Leu Leu Ala Cys Arg Asp Asp
    45                  50                  55

AAT TTG GTT TCA CAG CTT GTC CAT AGC CTC AAC CAG GTA TCA ACA GAT       602
Asn Leu Val Ser Gln Leu Val His Ser Leu Asn Gln Val Ser Thr Asp
60                  65                  70                  75

CAC ATA GAG TTG AAA GAT AAC CTT GGC AGT GAT GAC CCA GAA GGT GAC       650
His Ile Glu Leu Lys Asp Asn Leu Gly Ser Asp Asp Pro Glu Gly Asp
                80                  85                  90

ATA CCA GTC TTG TTG CAG GCC GTC CTG GCA AGG AGT CCT AAT GTT TTC       698
Ile Pro Val Leu Leu Gln Ala Val Leu Ala Arg Ser Pro Asn Val Phe
            95                  100                 105

AGG GAG AAA AGC ATG CAG AAC AGA TAT GTA CAA AGT GGA ATG ATG ATG       746
Arg Glu Lys Ser Met Gln Asn Arg Tyr Val Gln Ser Gly Met Met Met
        110                 115                 120

TCT CAG TAT AAA CTT TCT CAG AAT TCC ATG CAC AGT AGT CCT GCA TCT       794
Ser Gln Tyr Lys Leu Ser Gln Asn Ser Met His Ser Ser Pro Ala Ser
    125                 130                 135

TCC AAT TAT CAA CAA ACC ACT ATC TCA CAT AGC CCC TCC AGC CGG TTT       842
Ser Asn Tyr Gln Gln Thr Thr Ile Ser His Ser Pro Ser Ser Arg Phe
140                 145                 150                 155

GTG CCA CCA CAG ACA AGC TCT GGG AAC AGA TTT ATG CCA CAG CAA AAT       890
Val Pro Pro Gln Thr Ser Ser Gly Asn Arg Phe Met Pro Gln Gln Asn
                160                 165                 170

AGC CCA GTG CCT AGT CCA TAC GCC CCA CAA AGC CCT GCA GGA TAC ATG       938
Ser Pro Val Pro Ser Pro Tyr Ala Pro Gln Ser Pro Ala Gly Tyr Met
            175                 180                 185

CCA TAT TCC CAT CCT TCA AGT TAC ACA ACA CAT CCA CAG ATG CAA CAA       986
Pro Tyr Ser His Pro Ser Ser Tyr Thr Thr His Pro Gln Met Gln Gln
        190                 195                 200
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GCA TCG GTA TCA AGT CCC ATT GTT GCA GGT GGT TTG AGA AAC ATA CAT     1034
Ala Ser Val Ser Ser Pro Ile Val Ala Gly Gly Leu Arg Asn Ile His
    205                 210                 215

GAT AAT AAA GTT TCT GGT CCG TTG TCT GGC AAT TCA GCT AAT CAT CAT     1082
Asp Asn Lys Val Ser Gly Pro Leu Ser Gly Asn Ser Ala Asn His His
220                 225                 230                 235

GCT GAT AAT CCT AGA CAT GGT TCA AGT GAG GAC TAC CTA CAC ATG GTG     1130
Ala Asp Asn Pro Arg His Gly Ser Ser Glu Asp Tyr Leu His Met Val
                240                 245                 250

CAC AGG CTA AGT AGT GAC GAT GGA GAT TCT TCA ACA ATG AGG AAT GCT     1178
His Arg Leu Ser Ser Asp Asp Gly Asp Ser Ser Thr Met Arg Asn Ala
            255                 260                 265

GCA TCT TTT CCC TTG AGA TCT CCA CAG CCA GTA TGC TCC CCT GCT GGA     1226
Ala Ser Phe Pro Leu Arg Ser Pro Gln Pro Val Cys Ser Pro Ala Gly
        270                 275                 280

AGT GAA GGA ACT CCT AAA GGC TCA AGA CCA CCT TTA ATC CTA CAA TCT     1274
Ser Glu Gly Thr Pro Lys Gly Ser Arg Pro Pro Leu Ile Leu Gln Ser
    285                 290                 295

CAG TCT CTA CCT TGT TCA TCA CCT CGA GAT GTT CCA CCA GAT ATC TTG     1322
Gln Ser Leu Pro Cys Ser Ser Pro Arg Asp Val Pro Pro Asp Ile Leu
300                 305                 310                 315

CTA GAT TCT CCA GAA AGA AAA CAA AAG AAG CAG AAG AAA ATG AAA TTA     1370
Leu Asp Ser Pro Glu Arg Lys Gln Lys Lys Gln Lys Lys Met Lys Leu
                320                 325                 330

GGC AAG GAT GAA AAA GAG CAG AGT GAG AAA GCG GCA ATG TAT GAT ATA     1418
Gly Lys Asp Glu Lys Glu Gln Ser Glu Lys Ala Ala Met Tyr Asp Ile
            335                 340                 345

ATT AGT TCT CCA TCC AAG GAC TCT ACT AAA CTT ACA TTA AGA CTT TCT     1466
Ile Ser Ser Pro Ser Lys Asp Ser Thr Lys Leu Thr Leu Arg Leu Ser
        350                 355                 360

CGT GTA AGG TCT TCA GAC ATG GAC CAG CAA GAG GAT ATG ATT TCT GGT     1514
Arg Val Arg Ser Ser Asp Met Asp Gln Gln Glu Asp Met Ile Ser Gly
    365                 370                 375

GTG GAA AAT AGC AAT GTT TCA GAA AAT GAT ATT CCT TTT AAT GTG CAG     1562
Val Glu Asn Ser Asn Val Ser Glu Asn Asp Ile Pro Phe Asn Val Gln
380                 385                 390                 395

TAC CCA GGA CAG ACT TCA AAA ACA CCC ATT ACT CCA CAA GAT ATA AAC     1610
Tyr Pro Gly Gln Thr Ser Lys Thr Pro Ile Thr Pro Gln Asp Ile Asn
                400                 405                 410

CGC CCA CTA AAT GCT GCT CAA TGT TTG TCG CAG CAA GAA CAA ACA GCA     1658
Arg Pro Leu Asn Ala Ala Gln Cys Leu Ser Gln Gln Glu Gln Thr Ala
            415                 420                 425

TTC CTT CCA GCA AAT CAA GTG CCT GTT TTA CAA CAG AAC ACT TCA GTT     1706
Phe Leu Pro Ala Asn Gln Val Pro Val Leu Gln Gln Asn Thr Ser Val
        430                 435                 440

GCT GCA AAA CAA CCC CAG ACC AAT AGT CAC AAA ACC TTG GTG CAG CCT     1754
Ala Ala Lys Gln Pro Gln Thr Asn Ser His Lys Thr Leu Val Gln Pro
    445                 450                 455

GGA ACA GGC ATA GAG GTC TCA GCA GAG CTG CCC AAG GAC AAG ACC TAAGA   1804
Gly Thr Gly Ile Glu Val Ser Ala Glu Leu Pro Lys Asp Lys Thr
460                 465                 470

TCCAGCAGGG AACTATGTAG TCACCCCGAG AGGCCCAGCT CTCTCCGTGA GCTCTGGGCC   1864
TAGGGTGGGG GTGGTTGTTG GTTCTGCGCG CACTGTTCCC CCTACATGAT GGGTCCATCC   1924
CAGTTGGCTT CTCTCACTCG CTTCCTCCTG TGGAGAAGCC TGTCCAGGTG TCACTGCCTC   1984
CAGGAAGCTG TCTCTGATTT CTCCAGTTGA ACAGTGAGAT TTGCCACACC TCACATGCAT   2044
CGCTCTTGTC CCTGGAATTG TAACCATAGG TTTTCCTGTC TCCTGGAGGA CAAGGATGAG   2104
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GGCTTTCCAC TTGAGTCTCC CTGGTGGAGC CCAGCTCCTG ACATACCTGG TAAAAGTTCT   2164
CAAGAGAAGA ACATGGAGGA GGAATGTGGA TAACAACCCT GGCTGCCTGT GTGTTCCAAG   2224
CTAGGAAGAT GTAATGTCCC CACAAACGGG GTAAATGGCT TGCCTGCGTC ACAGCTGTCT   2284
CAAGCCCAGG CCCTGGGCGC CAGCCCAAGC CCAAGGACTA GGTCCAGAGC CACACAGCGC   2344
CAGGCCACAT CCGCCTCACC TGGGACCCTT TGTGGGGTAC AGTCTCCGGC CCCACCCAGA   2404
CCTCCTGAAG GAGAGACCCC ATGGCAAGGA CTCAGCCACC TGCAGTTTCA TAAGCCCCCA   2464
GTGGGTTCCT AGGCATGAAG ACCACCGGTT AGAGGCTGAA CTGGCAGGAA CCTGTCTCCA   2524
GCCCCTTCTC ACCCCAGCCG GGCCCTGCCT CAGAGGCAGC ACCCAGGACG TGGCCATGAC   2584
CCGTGGACTC CACTCAATCC CTCTTCTCCA GGAGCCATGC AAAGTGTCAG CCAGCCAGGC   2644
CCCTGGAAGG CAGTCATCAC CTCTTAAGGC ATTGTGGGTG TCGGTCCTGC AACTGCCAGG   2704
TGCAGCACAC GACCCGTGTC CGGTGTTCGA TAGCAGGGAG CCATGACCTG GCAACGATTC   2764
CACGCTCAAA GGGGCACCCG GGGGGCCCTG GGTCGGGGCG GATCAGCTTT CCCTGGGCAC   2824
ATCTGCCTCA TTCCAGATCT CCAGGGCTCA TGTCTGTGAC AGGGAGGGAA GGCTCTGCCC   2884
TGGCCTTCCG TCAGCTCTGC CAGTGCAGGC TGGGCAGCCT GGGCTTTAGA GCTGGCTTCT   2944
GCCCACACTT TCTCCGTGAA AGGAAAACAA CTATGAGTCT GCCAAACGCA TCTCAGATGC   3004
GTTTTAAAAA ATTCTGGTCC CCGCTCTCTG TCCCATCATC CGCCTCGGGG ACTTCCTCTC   3064
TCCGTGGTTC TCACCCCATA CTCTGTCACT GCCACATTTT CACCTGGGCC TGGCCTTTGT   3124
CTCCACCTGA AACTCCTGAA AATCTTGAAA TGGATTTCTA GGTCACTGGG GACTCCGGCA   3184
GCACATTCGG CTTCAGAATA AAGGGCGCCC GCGGTCCCCC AGCACCTCCC CAAGCCACAC   3244
CCCTAGCTTC CCTCCCTATC CCTGCAGCCT GAGGGTCCCT TCAGCCACCC TTAAGTCCCC   3304
ACCTGGGCTC CTGCCCCGCC CCTGGCTAGC AGCGCCTTCT CCACCGGGGC CCCCTCTGCT   3364
CACAGAGCCC CCTCACCTCC CTGGGGATGA GGGGCCAGGC CATGACCCTG AAAGTCTAGC   3424
CCTGGCCTTG ACCTCCCAGG AGCGCCCTCC CCGCCCTCTC CCGGCCCCGG CCCCGTCCTC   3484
TGCTGCTGGC CTCTGGGTCG TGCCCCGCAG ACTGAGCTGC GCTTGGGGGT CCTGGCGGCC   3544
TGGGCCGTCC CGCACCGAAC CCAGGCGGTC GGAGCCCGGC GGGAAGGCGC GAGGTCCTTC   3604
TGGGGGCTCC TCCGACGCCT GAGGGCGCTG CTTCCCCGCG GCCGCCCCGG GTTTCTGCGG   3664
AGCCGGGGCC TCCGCTCTCG GGTGACCCGG TGAGACCCCC GGGGAGGCCG CTGGGGAGGC   3724
GCGGGCTCTG CTCCCGGGTC CCAAACGCAC TGGCTGCCCC TCAGGAGGGA CGGCGACCTC   3784
CACCCACGGC GCTGGCGCCC GCACGGCCGC TCCTCCCGCT CCCGCAGCCT GGACGCCTCC   3844
CGAGGCCGCC CCGCCGGGCC CCACGCGCGG CCCCATCCGC AGGCCAGGAC TGCCTTCCCG   3904
GAGCTGGCGG CCCCCAGCCT GGAGGAGCCG GCCCCAGACG CCCTCCCAGC CCTCCCCAGC   3964
CCACTCTGGC CCCGCAGCCC CCGCCTGGTC CGAGTGCGGG TCTCTGGCCC CGGCCTTTCC   4024
CGGGGAAGGA AAGCAAAAAG CTT                                           4047
```

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 474 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

```
Met Asn Gly Asp Met Pro His Val Pro Ile Thr Thr Leu Ala Gly Ile
 1               5                  10                  15
Ala Ser Leu Thr Asp Leu Leu Asn Gln Leu Pro Leu Pro Ser Pro Leu
            20                  25                  30
Pro Ala Thr Thr Thr Lys Ser Leu Leu Phe Asn Ala Arg Ile Ala Glu
        35                  40                  45
Glu Val Asn Cys Leu Leu Ala Cys Arg Asp Asp Asn Leu Val Ser Gln
    50                  55                  60
Leu Val His Ser Leu Asn Gln Val Ser Thr Asp His Ile Glu Leu Lys
65                  70                  75                  80
Asp Asn Leu Gly Ser Asp Asp Pro Glu Gly Asp Ile Pro Val Leu Leu
                85                  90                  95
Gln Ala Val Leu Ala Arg Ser Pro Asn Val Phe Arg Glu Lys Ser Met
            100                 105                 110
Gln Asn Arg Tyr Val Gln Ser Gly Met Met Met Ser Gln Tyr Lys Leu
        115                 120                 125
Ser Gln Asn Ser Met His Ser Ser Pro Ala Ser Ser Asn Tyr Gln Gln
    130                 135                 140
Thr Thr Ile Ser His Ser Pro Ser Ser Arg Phe Val Pro Pro Gln Thr
145                 150                 155                 160
Ser Ser Gly Asn Arg Phe Met Pro Gln Gln Asn Ser Pro Val Pro Ser
                165                 170                 175
Pro Tyr Ala Pro Gln Ser Pro Ala Gly Tyr Met Pro Tyr Ser His Pro
            180                 185                 190
Ser Ser Tyr Thr Thr His Pro Gln Met Gln Gln Ala Ser Val Ser Ser
        195                 200                 205
```

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Pro Ile Val Ala Gly Gly Leu Arg Asn Ile His Asp Asn Lys Val Ser
    210                 215                 220
Gly Pro Leu Ser Gly Asn Ser Ala Asn His His Ala Asp Asn Pro Arg
225                 230                 235                 240
His Gly Ser Ser Glu Asp Tyr Leu His Met Val His Arg Leu Ser Ser
                245                 250                 255
Asp Asp Gly Asp Ser Ser Thr Met Arg Asn Ala Ala Ser Phe Pro Leu
            260                 265                 270
Arg Ser Pro Gln Pro Val Cys Ser Pro Ala Gly Ser Glu Gly Thr Pro
        275                 280                 285
Lys Gly Ser Arg Pro Pro Leu Ile Leu Gln Ser Gln Ser Leu Pro Cys
    290                 295                 300
Ser Ser Pro Arg Asp Val Pro Pro Asp Ile Leu Leu Asp Ser Pro Glu
305                 310                 315                 320
Arg Lys Gln Lys Lys Gln Lys Lys Met Lys Leu Gly Lys Asp Glu Lys
                325                 330                 335
Glu Gln Ser Glu Lys Ala Ala Met Tyr Asp Ile Ile Ser Ser Pro Ser
            340                 345                 350
Lys Asp Ser Thr Lys Leu Thr Leu Arg Leu Ser Arg Val Arg Ser Ser
        355                 360                 365
Asp Met Asp Gln Gln Glu Asp Met Ile Ser Gly Val Glu Asn Ser Asn
    370                 375                 380
Val Ser Glu Asn Asp Ile Pro Phe Asn Val Gln Tyr Pro Gly Gln Thr
385                 390                 395                 400
Ser Lys Thr Pro Ile Thr Pro Gln Asp Ile Asn Arg Pro Leu Asn Ala
                405                 410                 415
Ala Gln Cys Leu Ser Gln Gln Glu Gln Thr Ala Phe Leu Pro Ala Asn
            420                 425                 430
Gln Val Pro Val Leu Gln Gln Asn Thr Ser Val Ala Ala Lys Gln Pro
        435                 440                 445
Gln Thr Asn Ser His Lys Thr Leu Val Gln Pro Gly Thr Gly Ile Glu
    450                 455                 460
Val Ser Ala Glu Leu Pro Lys Asp Lys Thr
465                 470
```

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2998 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 26...799
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

```
AAGCTTTTTG AATTCGGCAC GAGAT GCT ACA CAG GCT ATA TTT GAA ATA CTG      52
                              Ala Thr Gln Ala Ile Phe Glu Ile Leu
                               1               5

GAG AAA TCC TGG TTG CCC CAG AAT TGT ACA CTG GTT GAT ATG AAG ATT     100
Glu Lys Ser Trp Leu Pro Gln Asn Cys Thr Leu Val Asp Met Lys Ile
10                  15                  20                  25

GAA TTT GGT GTT GAT GTA ACC ACC AAA GAA ATT GTT CTT GCT GAT GTT     148
Glu Phe Gly Val Asp Val Thr Thr Lys Glu Ile Val Leu Ala Asp Val
                30                  35                  40

ATT GAC AAT GAT TCC TGG AGA CTC TGG CCA TCA GGA GAT CGA AGC CAA     196
Ile Asp Asn Asp Ser Trp Arg Leu Trp Pro Ser Gly Asp Arg Ser Gln
            45                  50                  55

CAG AAA GAC AAA CAG TCT TAT CGG GAC CTC AAA GAA GTA ACT CCT GAA     244
Gln Lys Asp Lys Gln Ser Tyr Arg Asp Leu Lys Glu Val Thr Pro Glu
        60                  65                  70

GGG CTC CAA ATG GTA AAG AAA AAC TTT GAG TGG GTT GCA GAG AGA GTA     292
```

```
Gly Leu Gln Met Val Lys Lys Asn Phe Glu Trp Val Ala Glu Arg Val
    75                  80                  85

GAG TTG CTT TTG AAA TCA GAA AGT CAG TGC AGG GTT GTA GTG TTG ATG     340
Glu Leu Leu Leu Lys Ser Glu Ser Gln Cys Arg Val Val Val Leu Met
90                  95                  100                 105

GGC TCT ACT TCT GAT CTT GGT CAC TGT GAA AAA ATC AAG AAG GCC TGT     388
Gly Ser Thr Ser Asp Leu Gly His Cys Glu Lys Ile Lys Lys Ala Cys
                110                 115                 120

GGA AAT TTT GGC ATT CCA TGT GAA CTT CGA GTA ACA TCT GCG CAT AAA     436
Gly Asn Phe Gly Ile Pro Cys Glu Leu Arg Val Thr Ser Ala His Lys
            125                 130                 135

GGA CCA GAT GAA ACT CTG AGG ATT AAA GCT GAG TAT GAA GGG GAT GGC     484
Gly Pro Asp Glu Thr Leu Arg Ile Lys Ala Glu Tyr Glu Gly Asp Gly
        140                 145                 150

ATT CCT ACT GTA TTT GTG GCA GTG GCA GGC AGA AGT AAT GGT TTG GGA     532
Ile Pro Thr Val Phe Val Ala Val Ala Gly Arg Ser Asn Gly Leu Gly
    155                 160                 165

CCA GTG ATG TCT GGG AAC ACT GCA TAT CCA GTT ATC AGC TGT CCT CCC     580
Pro Val Met Ser Gly Asn Thr Ala Tyr Pro Val Ile Ser Cys Pro Pro
170                 175                 180                 185

CTC ACA CCA GAC TGG GGA GTT CAG GAT GTG TGG TCT TCT CTT CGA CTA     628
Leu Thr Pro Asp Trp Gly Val Gln Asp Val Trp Ser Ser Leu Arg Leu
                190                 195                 200

CCC AGT GGT CTT GGC TGT TCA ACC GTA CTT TCT CCA GAA GGA TCA GCT     676
Pro Ser Gly Leu Gly Cys Ser Thr Val Leu Ser Pro Glu Gly Ser Ala
            205                 210                 215

CAA TTT GCT GCT CAG ATA TTT GGG TTA AGC AAC CAT TTG GTA TGG AGC     724
Gln Phe Ala Ala Gln Ile Phe Gly Leu Ser Asn His Leu Val Trp Ser
        220                 225                 230

AAA CTG CGA GCA AGC ATT TTG AAC ACA TGG ATT TCC TTG AAG CAG GCT     772
Lys Leu Arg Ala Ser Ile Leu Asn Thr Trp Ile Ser Leu Lys Gln Ala
    235                 240                 245

GAC AAG AAA ATC AGA GAA TGT AAT TTA TAAGAAAGAA TGCCATTGAA TTTTTTA   826
Asp Lys Lys Ile Arg Glu Cys Asn Leu
250                 255

GGGGAAAAAC TACAAATTTC TAATTTAGCT GAAGGAAAAT CAAGCAAGAT GAAAAGGTAA    886
TTTTAAATTA GAGAACACAA ATAAAATGTA TTAGTGAATA AATGGTGAGG GTAGGCCTAT    946
TCAGATGCAA GGCCAGCAAT GGGGCTCCCC ATTATCCCCA CCCCTTTGGT CCCAGTCCCC   1006
TTCTCTGCAA TGGGCACGCA TAGAGGAGAG ACAAAGGGTA TTAGACGCAA CATCATTGGC   1066
CCAGGGGAGT CCGAGAAGAG CTGCCATTGG CTGACAGGGC ATTTTCAGGC TCTGTCATTG   1126
GTCAGGGAGC ACACCCCAGC CTGAAGAGTG ATGCCATTGG CCAGGGAGTG GTTTTGTCAT   1186
AGCCGTTGGC TGTGAAGTGG AAGGAAAAGA TCTGGGAATG AAGCCCTGTG GCCAGGAAGA   1246
TAGACAGGGC AGCAACTTCT GGGCCTCCAG GCCCTCTTCC CACCATAGCA ATGTGGGCAA   1306
AACTGGTGTC AGGCCCCAGC CAGAAAAAGG AGCCCAAGCC AGAGGGCAAG TGACAAAGGA   1366
TGTACCATGT CCAATCTCCC ACACCCTGGG GCTGCCCTTC CCAATGTCTT TCTTGATAGC   1426
CAAGTTGGGC TGGGAGCAGC TCACTGCTCC TCTAGCCAGG AGGGTTTCTC AGCTCCTGGA   1486
GGCCGCAGCT TGATGTTGAA CTGCTGCAGG GTCTGCTCCA GCTGTTTCTG GTTCCCAGCA   1546
AAGTAGGCGG ACACAGCATT GTGGAAGAGC AGCAGCTGCT TGTGCATCAC CTTGATCTTG   1606
TTTTCTTCCA GGAACTTGAG CTTGATGGCC ACATCTCCCC GCAGCTTCTC ATACTTGTCC   1666
CGATGGGCCT GGAAAGTGGC CTGGGCACTC TCAAGTCGAC CACGTGTCCC TGCATCCCGG   1726
GGGCCTAGAC TCAGCTCCTC TAAGTCTGTT CGGTAGGCAT CATATTCCAG CCTGGCAGCC   1786
TCATACTGTT TCACAGTCAT GAGCGTGTCT TCCATGGTCT TGGTGACCAA TGTGTTGATG   1846
CTAGAGACAA AGAAGTTCAC GGCTCCTAGC AGCGTTTCCC CATTCTTGCA TAGTAGTTTC   1906
TGTGTCTCTG CATTGTAGCC AAATTCCTCC TGAAGCTCTG GGGACTTCTG GCTGAGGTCA   1966
GCAAAGGCAT CACCCAGTGC ATGCTGGGTC TGCAGCAGGC TGTAGAGGTG GGCTGTCAGT   2026
GCCCGGCCCA GCTGCAGGAC ACTCTCATAC TTGCGCTTCG TCTCACGCAG CAACTCAATC   2086
TGCAGCTCTA GCTCCAGGAT TCCGGCGCCT CCACTCCGTC CCCCGCGGGT CTGCTCTGTG   2146
TGCCATGGAC GGCATTGTCC CAGATATAGC CGTTGGTACA AAGCGGGGAT CTGACGAGCT   2206
TTTCTCTACT TGTGTCACTA ACGGACCGTT TATCATGAGC AGCAACTCGG CTTCTGCAGC   2266
AAACGGAAAT GACAGCAAGA AGTTCAAAGG TGACAGCCGA AGTGCAGGCG TCCCCTCTAG   2326
AGTGATCCAC ATCCGGAAGC TCCCCATCGA CGTCACGGAG GGGGAAGTCA TCTCCCTGGG   2386
```

```
GCTGCCCTTT GGGAAGGTCA CCAACCTCCT GATGCTGAAG GGGAAAAACC AGGCCTTCAT   2446
CGAGATGAAC ACGGAGGAGG CTGCCAATAC CATGGTGAAC TACTACACCT CGGTGACCCC   2506
TGTGCTGCGC GGCCAGCCCA TCTACATCCA GTTCTCCAAC CACAAGGAGC TGAAGACCGA   2566
CAGCTCTCCC AACCAGGCGC GGGCCCAGGC GGCCCTGCAG GCGGTGAACT CGGTCCAGTC   2626
GGGGAACCTG GCCTTGGCTG CCTCGGCGGC GGCCGTGGAT GCAGGGATGG CGATGGCCGG   2686
GCAGAGCCCC GTGCTCAGGA TCATCGTGGA GAACCTCTTC TACCCTGTGA CCCTGGATGT   2746
GCTGCACCAG ATTTTCTCCA AGTTCGGCAC AGTGTTGAAG ATCATCACCT TCACCAAGAA   2806
CAACCAGTTC CAGGCCCTGC TGCAGTATGC GGACCCCGTG AGCGCCCAGC ACGCCAAGCT   2866
GTCGCTGGAC GGGCAGAACA TCTACAACGC CTGCTGCACG CTGCGCATCG ACTTTTCCAA   2926
GCTCACCAGC CTCAACGTCA AGTACAACAA TGACAAGAGC CGTGACTACC TCGTGCCGAA   2986
TTCTTTGGAT CC                                                      2998
```

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 258 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

```
Ala Thr Gln Ala Ile Phe Glu Ile Leu Glu Lys Ser Trp Leu Pro Gln
 1               5                  10                  15
Asn Cys Thr Leu Val Asp Met Lys Ile Glu Phe Gly Val Asp Val Thr
            20                  25                  30
Thr Lys Glu Ile Val Leu Ala Asp Val Ile Asp Asn Asp Ser Trp Arg
        35                  40                  45
Leu Trp Pro Ser Gly Asp Arg Ser Gln Gln Lys Asp Lys Gln Ser Tyr
    50                  55                  60
Arg Asp Leu Lys Glu Val Thr Pro Glu Gly Leu Gln Met Val Lys Lys
65                  70                  75                  80
Asn Phe Glu Trp Val Ala Glu Arg Val Glu Leu Leu Leu Lys Ser Glu
                85                  90                  95
Ser Gln Cys Arg Val Val Val Leu Met Gly Ser Thr Ser Asp Leu Gly
            100                 105                 110
His Cys Glu Lys Ile Lys Lys Ala Cys Gly Asn Phe Gly Ile Pro Cys
        115                 120                 125
Glu Leu Arg Val Thr Ser Ala His Lys Gly Pro Asp Glu Thr Leu Arg
    130                 135                 140
Ile Lys Ala Glu Tyr Glu Gly Asp Gly Ile Pro Thr Val Phe Val Ala
145                 150                 155                 160
Val Ala Gly Arg Ser Asn Gly Leu Gly Pro Val Met Ser Gly Asn Thr
                165                 170                 175
Ala Tyr Pro Val Ile Ser Cys Pro Pro Leu Thr Pro Asp Trp Gly Val
            180                 185                 190
Gln Asp Val Trp Ser Ser Leu Arg Leu Pro Ser Gly Leu Gly Cys Ser
        195                 200                 205
Thr Val Leu Ser Pro Glu Gly Ser Ala Gln Phe Ala Ala Gln Ile Phe
    210                 215                 220
Gly Leu Ser Asn His Leu Val Trp Ser Lys Leu Arg Ala Ser Ile Leu
225                 230                 235                 240
Asn Thr Trp Ile Ser Leu Lys Gln Ala Asp Lys Lys Ile Arg Glu Cys
                245                 250                 255
Asn Leu
```

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1038 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

```
Ile Gln Arg Phe Gly Thr Ser Gly His Ile Met Asn Leu Gln Ala Gln
 1               5                  10                  15
```

```
Pro Lys Ala Gln Asn Lys Arg Lys Arg Cys Leu Phe Gly Gly Gln Glu
            20                  25                  30
Pro Ala Pro Lys Glu Gln Pro Pro Pro Leu Gln Pro Pro Gln Gln Ser
        35                  40                  45
Ile Arg Val Lys Glu Glu Gln Tyr Leu Gly His Glu Gly Pro Gly Gly
    50                  55                  60
Ala Val Ser Thr Ser Gln Pro Val Glu Leu Pro Pro Pro Ser Ser Leu
65                  70                  75                  80
Ala Leu Leu Asn Ser Val Val Tyr Gly Pro Glu Arg Thr Ser Ala Ala
                85                  90                  95
Met Leu Ser Gln Gln Val Ala Ser Val Lys Trp Pro Asn Ser Val Met
            100                 105                 110
Ala Pro Gly Arg Gly Pro Glu Arg Gly Gly Gly Gly Gly Val Ser Asp
        115                 120                 125
Ser Ser Trp Gln Gln Gln Pro Gly Gln Pro Pro Pro His Ser Thr Trp
    130                 135                 140
Asn Cys His Ser Leu Ser Leu Tyr Ser Ala Thr Lys Gly Ser Pro His
145                 150                 155                 160
Pro Gly Val Gly Val Pro Thr Tyr Tyr Asn His Pro Glu Ala Leu Lys
                165                 170                 175
Arg Glu Lys Ala Gly Gly Pro Gln Leu Asp Arg Tyr Val Arg Pro Met
            180                 185                 190
Met Pro Gln Lys Val Gln Leu Glu Val Gly Arg Pro Gln Ala Pro Leu
        195                 200                 205
Asn Ser Phe His Ala Ala Lys Lys Pro Pro Asn Gln Ser Leu Pro Leu
    210                 215                 220
Gln Pro Phe Gln Leu Ala Phe Gly His Gln Val Asn Arg Gln Val Phe
225                 230                 235                 240
Arg Gln Gly Pro Pro Pro Pro Asn Pro Val Ala Ala Phe Pro Pro Gln
                245                 250                 255
Lys Gln Gln Gln Gln Gln Gln Pro Gln Gln Gln Gln Gln Gln Gln Gln
            260                 265                 270
Ala Ala Leu Pro Gln Met Pro Leu Phe Glu Asn Phe Tyr Ser Met Pro
        275                 280                 285
Gln Gln Pro Ser Gln Gln Pro Gln Asp Phe Gly Leu Gln Pro Ala Gly
    290                 295                 300
Pro Leu Gly Gln Ser His Leu Ala His His Ser Met Ala Pro Tyr Pro
305                 310                 315                 320
Phe Pro Pro Asn Pro Asp Met Asn Pro Glu Leu Arg Lys Ala Leu Leu
                325                 330                 335
Gln Asp Ser Ala Pro Gln Pro Ala Leu Pro Gln Val Gln Ile Pro Phe
            340                 345                 350
Pro Arg Arg Ser Arg Arg Leu Ser Lys Glu Gly Ile Leu Pro Pro Ser
        355                 360                 365
Ala Leu Asp Gly Ala Gly Thr Gln Pro Gly Gln Glu Ala Thr Gly Asn
    370                 375                 380
Leu Phe Leu His His Trp Pro Leu Gln Gln Pro Pro Pro Gly Ser Leu
385                 390                 395                 400
Gly Gln Pro His Pro Glu Ala Leu Gly Phe Pro Leu Glu Leu Arg Glu
                405                 410                 415
Ser Gln Leu Leu Pro Asp Gly Glu Arg Leu Ala Pro Asn Gly Arg Glu
            420                 425                 430
Arg Glu Ala Pro Ala Met Gly Ser Glu Glu Gly Met Arg Ala Val Ser
        435                 440                 445
Thr Gly Asp Cys Gly Gln Val Leu Arg Gly Gly Val Ile Gln Ser Thr
    450                 455                 460
Arg Arg Arg Arg Arg Ala Ser Gln Glu Ala Asn Leu Leu Thr Leu Ala
465                 470                 475                 480
Gln Lys Ala Val Glu Leu Ala Ser Leu Gln Asn Ala Lys Asp Gly Ser
                485                 490                 495
Gly Ser Glu Glu Lys Arg Lys Ser Val Leu Ala Ser Thr Thr Lys Cys
            500                 505                 510
Gly Val Glu Phe Ser Glu Pro Ser Leu Ala Thr Lys Arg Ala Arg Glu
        515                 520                 525
Asp Ser Gly Met Val Pro Leu Ile Ile Pro Val Ser Val Pro Val Arg
    530                 535                 540
Thr Val Asp Pro Thr Glu Ala Ala Gln Ala Gly Gly Leu Asp Glu Asp
545                 550                 555                 560
Gly Lys Gly Leu Glu Gln Asn Pro Ala Glu His Lys Pro Ser Val Ile
                565                 570                 575
Val Thr Arg Arg Arg Ser Thr Arg Ile Pro Gly Thr Asp Ala Gln Ala
            580                 585                 590
Gln Ala Glu Asp Met Asn Val Lys Leu Glu Gly Glu Pro Ser Val Arg
```

```
        595                 600                 605
Lys Pro Lys Gln Arg Pro Arg Pro Glu Pro Leu Ile Ile Pro Thr Lys
    610                 615                 620
Ala Gly Thr Phe Ile Ala Pro Pro Val Tyr Ser Asn Ile Thr Pro Tyr
625                 630                 635                 640
Gln Ser His Leu Arg Ser Pro Val Arg Leu Ala Asp His Pro Ser Glu
                645                 650                 655
Arg Ser Phe Glu Leu Pro Pro Tyr Thr Pro Pro Pro Ile Leu Ser Pro
            660                 665                 670
Val Arg Glu Gly Ser Gly Leu Tyr Phe Asn Ala Ile Ile Ser Thr Ser
        675                 680                 685
Thr Ile Pro Ala Pro Pro Pro Ile Thr Pro Lys Ser Ala His Arg Thr
    690                 695                 700
Leu Leu Arg Thr Asn Ser Ala Glu Val Thr Pro Pro Val Leu Ser Val
705                 710                 715                 720
Met Gly Glu Ala Thr Pro Val Ser Ile Glu Pro Arg Ile Asn Val Gly
                725                 730                 735
Ser Arg Phe Gln Ala Glu Ile Pro Leu Met Arg Asp Arg Ala Leu Ala
            740                 745                 750
Ala Ala Asp Pro His Lys Ala Asp Leu Val Trp Gln Pro Trp Glu Asp
        755                 760                 765
Leu Glu Ser Ser Arg Glu Lys Gln Arg Gln Val Glu Asp Leu Leu Thr
    770                 775                 780
Ala Ala Cys Ser Ser Ile Phe Pro Gly Ala Gly Thr Asn Gln Glu Leu
785                 790                 795                 800
Ala Leu His Cys Leu His Glu Ser Arg Gly Asp Ile Leu Glu Thr Leu
                805                 810                 815
Asn Lys Leu Leu Leu Lys Lys Pro Leu Arg Pro His Asn His Pro Leu
            820                 825                 830
Ala Thr Tyr His Tyr Thr Gly Ser Asp Gln Trp Lys Met Ala Glu Arg
        835                 840                 845
Lys Leu Phe Asn Lys Gly Ile Ala Ile Tyr Lys Lys Asp Phe Phe Leu
    850                 855                 860
Val Gln Lys Leu Ile Gln Thr Lys Thr Val Ala Gln Cys Val Glu Phe
865                 870                 875                 880
Tyr Tyr Thr Tyr Lys Lys Gln Val Lys Ile Gly Arg Asn Gly Thr Leu
                885                 890                 895
Thr Phe Gly Asp Val Asp Thr Ser Asp Glu Lys Ser Ala Gln Glu Glu
            900                 905                 910
Val Glu Val Asp Ile Lys Thr Ser Gln Lys Phe Pro Arg Val Pro Leu
        915                 920                 925
Pro Arg Arg Glu Ser Pro Ser Glu Glu Arg Leu Glu Pro Lys Arg Glu
    930                 935                 940
Val Lys Glu Pro Arg Lys Glu Gly Glu Glu Glu Val Pro Glu Ile Gln
945                 950                 955                 960
Glu Lys Glu Glu Gln Glu Glu Gly Arg Glu Arg Ser Arg Arg Ala Ala
                965                 970                 975
Ala Val Lys Ala Thr Gln Thr Leu Gln Ala Asn Glu Ser Ala Ser Asp
            980                 985                 990
Ile Leu Ile Leu Arg Ser His Glu Ser Asn Ala Pro Gly Ser Ala Gly
        995                 1000                1005
Gly Gln Ala Ser Glu Lys Pro Arg Glu Gly Thr Gly Lys Ser Arg Arg
    1010                1015                1020
Ala Leu Pro Phe Ser Glu Lys Lys Lys Lys Lys Gln Lys Ala
1025                1030                1035
```

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 849 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

```
Ile Arg His Glu Val Ser Phe Leu Trp Asn Thr Glu Ala Ala Cys Pro
 1               5                  10                  15
Ile Gln Thr Thr Thr Asp Thr Asp Gln Ala Cys Ser Ile Arg Asp Pro
            20                  25                  30
Asn Ser Gly Phe Val Phe Asn Leu Asn Pro Leu Asn Ser Ser Gln Gly
        35                  40                  45
```

```
Tyr Asn Val Ser Gly Ile Gly Lys Ile Phe Met Phe Asn Val Cys Gly
    50                  55                  60
Thr Met Pro Val Cys Gly Thr Ile Leu Gly Lys Pro Ala Ser Gly Cys
65                  70                  75                  80
Glu Ala Glu Thr Gln Thr Glu Glu Leu Lys Asn Trp Lys Pro Ala Arg
                85                  90                  95
Pro Val Gly Ile Glu Lys Ser Leu Gln Leu Ser Thr Glu Gly Phe Ile
            100                 105                 110
Thr Leu Thr Tyr Lys Gly Pro Leu Ser Ala Lys Gly Thr Ala Asp Ala
        115                 120                 125
Phe Ile Val Arg Phe Val Cys Asn Asp Asp Val Tyr Ser Gly Pro Leu
    130                 135                 140
Lys Phe Leu His Gln Asp Ile Asp Ser Gly Gln Gly Ile Arg Asn Thr
145                 150                 155                 160
Tyr Phe Glu Phe Glu Thr Ala Leu Ala Cys Val Pro Ser Pro Val Asp
                165                 170                 175
Cys Gln Val Thr Asp Leu Ala Gly Asn Glu Tyr Asp Leu Thr Gly Leu
            180                 185                 190
Ser Thr Val Arg Lys Pro Trp Thr Ala Val Asp Thr Ser Val Asp Gly
        195                 200                 205
Arg Lys Arg Thr Phe Tyr Leu Ser Val Cys Asn Pro Leu Pro Tyr Ile
    210                 215                 220
Pro Gly Cys Gln Gly Ser Ala Val Gly Ser Cys Leu Val Ser Glu Gly
225                 230                 235                 240
Asn Ser Trp Asn Leu Gly Val Val Gln Met Ser Pro Gln Ala Ala Ala
                245                 250                 255
Asn Gly Ser Leu Ser Ile Met Tyr Val Asn Gly Asp Lys Cys Gly Asn
            260                 265                 270
Gln Arg Phe Ser Thr Arg Ile Thr Phe Glu Cys Ala Gln Ile Ser Gly
        275                 280                 285
Ser Pro Ala Phe Gln Leu Gln Asp Gly Cys Glu Tyr Val Phe Ile Trp
    290                 295                 300
Arg Thr Val Glu Ala Cys Pro Val Val Arg Val Glu Gly Asp Asn Cys
305                 310                 315                 320
Glu Val Lys Asp Pro Arg His Gly Asn Leu Tyr Asp Leu Lys Pro Leu
                325                 330                 335
Gly Leu Asn Asp Thr Ile Val Ser Ala Gly Glu Tyr Thr Tyr Tyr Phe
            340                 345                 350
Arg Val Cys Gly Lys Leu Ser Ser Asp Val Cys Pro Thr Ser Asp Lys
        355                 360                 365
Ser Lys Val Val Ser Ser Cys Gln Glu Lys Arg Glu Pro Gln Gly Phe
    370                 375                 380
His Lys Val Ala Gly Leu Leu Thr Gln Lys Leu Thr Tyr Glu Asn Gly
385                 390                 395                 400
Leu Leu Lys Met Asn Phe Thr Gly Gly Asp Thr Cys His Lys Val Tyr
                405                 410                 415
Gln Arg Ser Thr Ala Ile Phe Phe Tyr Cys Asp Arg Gly Thr Gln Arg
            420                 425                 430
Pro Val Phe Leu Lys Glu Thr Ser Asp Cys Ser Tyr Leu Phe Glu Trp
        435                 440                 445
Arg Thr Gln Tyr Ala Cys Pro Pro Phe Asp Leu Thr Glu Cys Ser Phe
    450                 455                 460
Lys Asp Gly Ala Gly Asn Ser Phe Asp Leu Ser Ser Leu Ser Arg Tyr
465                 470                 475                 480
Ser Asp Asn Trp Glu Ala Ile Thr Gly Thr Gly Asp Pro Glu His Tyr
                485                 490                 495
Leu Ile Asn Val Cys Lys Ser Leu Ala Pro Gln Ala Gly Thr Glu Pro
            500                 505                 510
Cys Pro Pro Glu Ala Ala Ala Cys Leu Leu Gly Gly Ser Lys Pro Val
        515                 520                 525
Asn Leu Gly Arg Val Arg Asp Gly Pro Gln Trp Arg Asp Gly Ile Ile
    530                 535                 540
Val Leu Lys Tyr Val Asp Gly Asp Leu Cys Pro Asp Gly Ile Arg Lys
545                 550                 555                 560
Lys Ser Thr Thr Ile Arg Phe Thr Cys Ser Glu Ser Gln Val Asn Ser
                565                 570                 575
Arg Pro Met Phe Ile Ser Ala Val Glu Asp Cys Glu Tyr Thr Phe Ala
            580                 585                 590
Trp Pro Thr Ala Thr Ala Cys Pro Met Lys Ser Asn Glu His Asp Asp
        595                 600                 605
Cys Gln Val Thr Asn Pro Ser Thr Gly His Leu Phe Asp Leu Ser Ser
    610                 615                 620
Leu Ser Gly Arg Ala Gly Phe Thr Ala Ala Tyr Ser Glu Lys Gly Leu
```

625 630 635 640
Val Tyr Met Ser Ile Cys Gly Glu Asn Glu Asn Cys Pro Pro Gly Val
645 650 655
Gly Ala Cys Phe Gly Gln Thr Arg Ile Ser Val Gly Lys Ala Asn Lys
660 665 670
Arg Leu Arg Tyr Val Asp Gln Val Leu Gln Leu Val Tyr Lys Asp Gly
675 680 685
Ser Pro Cys Pro Ser Lys Ser Gly Leu Ser Tyr Lys Ser Val Ile Ser
690 695 700
Phe Val Cys Arg Pro Glu Ala Gly Pro Thr Asn Arg Pro Met Leu Ile
705 710 715 720
Ser Leu Asp Lys Gln Thr Cys Thr Leu Phe Phe Ser Trp His Thr Pro
725 730 735
Leu Ala Cys Glu Gln Ala Thr Glu Cys Ser Val Arg Asn Gly Ser Ser
740 745 750
Ile Val Asp Leu Ser Pro Leu Ile His Arg Thr Gly Gly Tyr Glu Ala
755 760 765
Tyr Asp Glu Ser Glu Asp Asp Ala Ser Asp Thr Asn Pro Asp Phe Tyr
770 775 780
Ile Asn Ile Cys Gln Pro Leu Asn Pro Met His Gly Val Pro Cys Pro
785 790 795 800
Ala Gly Ala Ala Val Cys Lys Val Pro Ile Asp Gly Pro Pro Ile Asp
805 810 815
Ile Gly Arg Val Ala Gly Pro Pro Ile Leu Asn Pro Ile Ala Asn Glu
820 825 830
Ile Tyr Leu Asn Phe Glu Ser Ser Thr Pro Cys Gln Glu Phe Ser Cys
835 840 845
Lys

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 852 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Met Ala Arg Leu Ser Arg Pro Glu Arg Pro Asp Leu Val Phe Glu Glu
1 5 10 15
Glu Asp Leu Pro Tyr Glu Glu Glu Ile Met Arg Asn Gln Phe Ser Val
20 25 30
Lys Cys Trp Leu His Tyr Ile Glu Phe Lys Gln Gly Ala Pro Lys Pro
35 40 45
Arg Leu Asn Gln Leu Tyr Glu Arg Ala Leu Lys Leu Leu Pro Cys Ser
50 55 60
Tyr Lys Leu Trp Tyr Arg Tyr Leu Lys Ala Arg Arg Ala Gln Val Lys
65 70 75 80
His Arg Cys Val Thr Asp Pro Ala Tyr Glu Asp Val Asn Asn Cys His
85 90 95
Glu Arg Ala Phe Val Phe Met His Lys Met Pro Arg Leu Trp Leu Asp
100 105 110
Tyr Cys Gln Phe Leu Met Asp Gln Gly Arg Val Thr His Thr Arg Arg
115 120 125
Thr Phe Asp Arg Ala Leu Arg Ala Leu Pro Ile Thr Gln His Ser Arg
130 135 140
Ile Trp Pro Leu Tyr Leu Arg Phe Leu Arg Ser His Pro Leu Pro Glu
145 150 155 160
Thr Ala Val Arg Gly Tyr Arg Arg Phe Leu Lys Leu Ser Pro Glu Ser
165 170 175
Ala Glu Glu Tyr Ile Glu Tyr Leu Lys Ser Ser Asp Arg Leu Asp Glu
180 185 190
Ala Ala Gln Arg Leu Ala Thr Val Val Asn Asp Glu Arg Phe Val Ser
195 200 205
Lys Ala Gly Lys Ser Asn Tyr Gln Leu Trp His Glu Leu Cys Asp Leu
210 215 220
Ile Ser Gln Asn Pro Asp Lys Val Gln Ser Leu Asn Val Asp Ala Ile
225 230 235 240
Ile Arg Gly Gly Leu Thr Arg Phe Thr Asp Gln Leu Gly Lys Leu Trp
245 250 255

```
Cys Ser Leu Ala Asp Tyr Tyr Ile Arg Ser Gly His Phe Glu Lys Ala
                260                 265                 270
Arg Asp Val Tyr Glu Glu Ala Ile Arg Thr Val Met Thr Val Arg Asp
            275                 280                 285
Phe Thr Gln Val Phe Asp Ser Tyr Ala Gln Phe Glu Glu Ser Met Ile
    290                 295                 300
Ala Ala Lys Met Glu Thr Ala Ser Glu Leu Gly Arg Glu Glu Glu Asp
305                 310                 315                 320
Asp Val Asp Leu Glu Leu Arg Leu Ala Arg Phe Glu Gln Leu Ile Ser
                325                 330                 335
Arg Arg Pro Leu Leu Leu Asn Ser Val Leu Leu Arg Gln Asn Pro His
            340                 345                 350
His Val His Glu Trp His Lys Arg Val Ala Leu His Gln Gly Arg Pro
        355                 360                 365
Arg Glu Ile Ile Asn Thr Tyr Thr Glu Ala Val Gln Thr Val Asp Pro
    370                 375                 380
Phe Lys Ala Thr Gly Lys Pro His Thr Leu Trp Val Ala Phe Ala Lys
385                 390                 395                 400
Phe Tyr Glu Asp Asn Gly Gln Leu Asp Asp Ala Arg Val Ile Leu Glu
                405                 410                 415
Lys Ala Thr Lys Val Asn Phe Lys Gln Val Asp Asp Leu Ala Ser Val
            420                 425                 430
Trp Cys Gln Cys Gly Glu Leu Glu Leu Arg His Glu Asn Tyr Asp Glu
        435                 440                 445
Ala Leu Arg Leu Leu Arg Lys Ala Thr Ala Leu Pro Ala Arg Arg Ala
    450                 455                 460
Glu Tyr Phe Asp Gly Ser Glu Pro Val Gln Asn Arg Val Tyr Lys Ser
465                 470                 475                 480
Leu Lys Val Trp Ser Met Leu Ala Asp Leu Glu Glu Ser Leu Gly Thr
                485                 490                 495
Phe Gln Ser Thr Lys Ala Val Tyr Asp Arg Ile Leu Asp Leu Arg Ile
            500                 505                 510
Ala Thr Pro Gln Ile Val Ile Asn Tyr Ala Met Phe Leu Glu Glu His
        515                 520                 525
Lys Tyr Phe Glu Glu Ser Phe Lys Ala Tyr Glu Arg Gly Ile Ser Leu
    530                 535                 540
Phe Lys Trp Pro Asn Val Ser Asp Ile Trp Ser Thr Tyr Leu Thr Lys
545                 550                 555                 560
Phe Ile Ala Arg Tyr Gly Gly Arg Lys Leu Glu Arg Ala Arg Asp Leu
                565                 570                 575
Phe Glu Gln Ala Leu Asp Gly Cys Pro Pro Lys Tyr Ala Lys Thr Leu
            580                 585                 590
Tyr Leu Leu Tyr Ala Gln Leu Glu Glu Glu Trp Gly Leu Ala Arg His
        595                 600                 605
Ala Met Ala Val Tyr Glu Arg Ala Thr Arg Ala Val Glu Pro Ala Gln
    610                 615                 620
Gln Tyr Asp Met Phe Asn Ile Tyr Ile Lys Arg Ala Ala Glu Ile Tyr
625                 630                 635                 640
Gly Val Thr His Thr Arg Gly Ile Tyr Gln Lys Ala Ile Glu Val Leu
                645                 650                 655
Ser Asp Glu His Ala Arg Glu Met Cys Leu Arg Phe Ala Asp Met Glu
            660                 665                 670
Cys Lys Leu Gly Glu Ile Asp Arg Ala Arg Ala Ile Tyr Ser Phe Cys
        675                 680                 685
Ser Gln Ile Cys Asp Pro Arg Thr Thr Gly Ala Phe Trp Gln Thr Trp
    690                 695                 700
Lys Asp Phe Glu Val Arg His Gly Asn Glu Asp Thr Ile Lys Glu Met
705                 710                 715                 720
Leu Arg Ile Arg Arg Ser Val Gln Ala Thr Tyr Asn Thr Gln Val Asn
                725                 730                 735
Phe Met Ala Ser Gln Met Leu Lys Val Ser Gly Ser Ala Thr Gly Thr
            740                 745                 750
Val Ser Asp Leu Ala Pro Gly Gln Ser Gly Met Asp Asp Met Lys Leu
        755                 760                 765
Leu Glu Gln Arg Ala Glu Gln Leu Ala Ala Glu Ala Glu Arg Asp Gln
    770                 775                 780
Pro Leu Arg Ala Gln Ser Lys Ile Leu Phe Val Arg Ser Asp Ala Ser
785                 790                 795                 800
Arg Glu Glu Leu Ala Glu Leu Ala Gln Gln Val Asn Pro Glu Glu Ile
                805                 810                 815
Gln Leu Gly Glu Asp Glu Asp Glu Asp Glu Met Asp Leu Glu Pro Asn
            820                 825                 830
Glu Val Arg Leu Glu Gln Gln Ser Val Pro Ala Ala Val Phe Gly Ser
```

```
        835                 840                 845
Leu Lys Glu Asp
    850
```

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 693 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

```
Met Phe Ser Ala Leu Lys Lys Leu Val Gly Ser Asp Gln Ala Pro Gly
 1               5                  10                  15
Arg Asp Lys Asn Ile Pro Ala Gly Leu Gln Ser Met Asn Gln Ala Leu
            20                  25                  30
Gln Arg Arg Phe Ala Lys Gly Val Gln Tyr Asn Met Lys Ile Val Ile
        35                  40                  45
Arg Gly Asp Arg Asn Thr Gly Lys Thr Ala Leu Trp His Arg Leu Gln
    50                  55                  60
Gly Arg Pro Phe Val Glu Glu Tyr Ile Pro Thr Gln Glu Ile Gln Val
65                  70                  75                  80
Thr Ser Ile His Trp Ser Tyr Lys Thr Thr Asp Asp Ile Val Lys Val
                85                  90                  95
Glu Val Trp Asp Val Val Asp Lys Gly Lys Cys Lys Lys Arg Gly Asp
            100                 105                 110
Gly Leu Lys Met Glu Asn Asp Pro Gln Glu Xaa Glu Ser Glu Met Ala
        115                 120                 125
Leu Asp Ala Glu Phe Leu Asp Val Tyr Lys Asn Cys Asn Gly Val Val
    130                 135                 140
Met Met Phe Asp Ile Thr Lys Gln Trp Thr Phe Asn Tyr Ile Leu Arg
145                 150                 155                 160
Glu Leu Pro Lys Val Pro Thr His Val Pro Val Cys Val Leu Gly Asn
                165                 170                 175
Tyr Arg Asp Met Gly Glu His Arg Val Ile Leu Pro Asp Asp Val Arg
            180                 185                 190
Asp Phe Ile Asp Asn Leu Asp Arg Pro Pro Gly Ser Ser Tyr Phe Arg
        195                 200                 205
Tyr Ala Glu Ser Ser Met Lys Asn Ser Phe Gly Leu Lys Tyr Leu His
    210                 215                 220
Lys Phe Phe Asn Ile Pro Phe Leu Gln Leu Gln Arg Glu Thr Leu Leu
225                 230                 235                 240
Arg Gln Leu Glu Thr Asn Gln Leu Asp Met Asp Ala Thr Leu Glu Glu
                245                 250                 255
Leu Ser Val Gln Gln Glu Thr Glu Asp Gln Asn Tyr Gly Ile Phe Leu
            260                 265                 270
Glu Met Met Glu Ala Arg Ser Arg Gly His Ala Ser Pro Leu Ala Ala
        275                 280                 285
Asn Gly Gln Ser Pro Ser Pro Gly Ser Gln Ser Pro Val Leu Pro Ala
    290                 295                 300
Pro Ala Val Ser Thr Gly Ser Ser Ser Pro Gly Thr Pro Gln Pro Ala
305                 310                 315                 320
Pro Gln Leu Pro Leu Asn Ala Ala Pro Pro Ser Ser Val Pro Pro Val
                325                 330                 335
Pro Pro Ser Glu Ala Leu Pro Pro Pro Ala Cys Pro Ser Ala Pro Ala
            340                 345                 350
Pro Arg Arg Ser Ile Ile Ser Arg Leu Phe Gly Thr Ser Pro Ala Thr
        355                 360                 365
Glu Ala Ala Pro Pro Pro Pro Glu Pro Val Pro Ala Ala Gln Gly Pro
    370                 375                 380
Ala Thr Val Gln Ser Val Glu Asp Phe Val Pro Asp Asp Arg Leu Asp
385                 390                 395                 400
Arg Ser Phe Leu Glu Asp Thr Thr Pro Ala Arg Asp Glu Lys Lys Val
                405                 410                 415
Gly Ala Lys Ala Ala Gln Gln Asp Ser Asp Ser Asp Gly Glu Ala Leu
            420                 425                 430
Gly Gly Asn Pro Met Val Ala Gly Phe Gln Asp Asp Val Asp Leu Glu
        435                 440                 445
Asp Gln Pro Arg Gly Ser Pro Pro Leu Pro Ala Gly Pro Val Pro Ser
    450                 455                 460
```

```
Gln Asp Ile Thr Leu Ser Ser Glu Glu Glu Ala Glu Val Ala Ala Pro
465                 470                 475                 480
Thr Lys Gly Pro Ala Pro Ala Pro Gln Gln Cys Ser Glu Pro Glu Thr
                485                 490                 495
Lys Trp Ser Ser Ile Pro Ala Ser Lys Pro Arg Arg Gly Thr Ala Pro
            500                 505                 510
Thr Arg Thr Ala Ala Pro Pro Trp Pro Gly Gly Val Ser Val Arg Thr
        515                 520                 525
Gly Pro Glu Lys Arg Ser Ser Thr Arg Pro Pro Ala Glu Met Glu Pro
    530                 535                 540
Gly Lys Gly Glu Gln Ala Ser Ser Ser Glu Ser Asp Pro Glu Gly Pro
545                 550                 555                 560
Ile Ala Ala Gln Met Leu Ser Phe Val Met Asp Asp Pro Asp Phe Glu
                565                 570                 575
Ser Glu Gly Ser Asp Thr Gln Arg Arg Ala Asp Asp Phe Pro Val Arg
            580                 585                 590
Asp Asp Pro Ser Asp Val Thr Asp Glu Asp Glu Gly Pro Ala Glu Pro
        595                 600                 605
Pro Pro Pro Pro Lys Leu Pro Leu Pro Ala Phe Arg Leu Lys Asn Asp
    610                 615                 620
Ser Asp Leu Phe Gly Leu Gly Leu Glu Glu Ala Gly Pro Lys Glu Ser
625                 630                 635                 640
Ser Glu Glu Gly Lys Glu Gly Lys Thr Pro Ser Lys Glu Lys Lys Lys
                645                 650                 655
Lys Thr Lys Ser Phe Ser Arg Val Leu Leu Glu Arg Pro Arg Ala His
            660                 665                 670
Arg Phe Ser Thr Arg Val Gly Tyr Gln Val Ser Val Pro Asn Ser Pro
        675                 680                 685
Tyr Ser Glu Ser Tyr
    690
```

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/57, 15/12, C07K 14/47,
(Continued on the following page)

A3

(11) International Publication Number: WO 99/58559

(43) International Publication Date: 18 November 1999 (18.11.99)

(21) International Application Number: PCT/US99/10793

(22) International Filing Date: 14 May 1999 (14.05.99)

(30) Priority Data:
09/081,385 14 May 1998 (14.05.98) US

(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GATANAGA, Tetsuya [JP/US]; 77 Wellesley, Irvine, CA 92612 (US). GRANGER, Gale, A. [US/US]; 31562 Santa Rosa, Laguna Beach, CA 92651 (US).

(74) Agents: CAMPBELL, Cathryn et al.; Campbell and Flores, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
20 January 2000 (20.01.00)

(54) Title: FACTORS AFFECTING TUMOR NECROSIS FACTOR RECEPTOR RELEASING ENZYME ACTIVITY

(57) Abstract

The biological effects of the cytokine TNF are mediated by binding to receptors on the surface of cells. This disclosure describes new proteins and polynucleotides that promote enzymatic cleavage and release of TNF receptors. Also provided are method for identifying additional compounds that influence TNF receptor shedding. As the active ingredient in a pharmaceutical composition, the products of this invention increase or decrease TNF signal transduction, thereby alleviating the pathology of disease.

C12N 9/64, 15/11, C07K 16/18, 16/40, C12Q 1/68, G01N 33/68, 33/573, C12Q 1/37, A61K 38/17, 38/48, 48/00, 39/395

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

[illegible]tional Application No
PCT/US 99/10793

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C12N15/12 C07K14/47 C12N9/64 C12N15/11
C07K16/18 C07K16/40 C12Q1/68 G01N33/68 G01N33/573
C12Q1/37 A61K38/17 A61K38/48 A61K48/00 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no AJ003355 Sequence reference HSJ003355 13 October 1997 SZULZEWSKY I ET AL: "An integrated transcript map for the whole human chromosome 21" XP002121991 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA779203 Sequence reference AA779203 6 February 1998 HILLIER L ETAL: "WashU-NCI human EST project" XP002122454 the whole document	3-5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 1999	03/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Van der Schaal, C

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no U52222 Sequence reference HS5252210 26 April 1996 KANOH H AND EXTON J: "Human afarpatin 2, a putative target protein of ADP-ribosilation" XP002122455 the whole document	1,7,9-17
X	MINET M AND LACROUTE F: "Cloning and sequencing of a human cDNA coding for a multifunctional polypeptide of the purine pathway by complememntation of the ade2-101 mutant in Saccharomyces cerevisiae" CURRENT GENETICS, vol. 18, 1990, pages 287-291, XP002122452 figure 3	1,7,9-17
X	GONZALEZ I ET AL: "Variation among human 28S ribosomal genes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 82, November 1985 (1985-11), pages 7666-7670, XP002122453 WASHINGTON US figure 3	1
X	EMBL/GENBANK DATABASES Accession no T33896 Sequence reference HS89620 14 January 1995 ADAMS M ET AL: "Initial assessment of human gene diversity and expression patters based upon 52 ...." XP002122456 the whole document	3-5
P,X	EMBL/GENBANK DATABASES Accession no AI002979 Sequence reference AI002979 11 June 1998 HILLIER L ET AL: "WashU-NCI human EST project" XP002122457 figure W	3-5
X	EMBL/GENBANK DATABASES Accession no AA806165 Sequence reference AA806165 16 February 1998 "National Cancer Insitiute, Cancer Genome Anatomy Project" XP002122458 the whole document	3-5

-/—

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OSHIMA A ET AL: "The human cation-independent mannose 6-phosphate receptor" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 263, no. 5, 15 February 1988 (1988-02-15), pages 2553-2562, XP002121990 MD US see figure 2 amino acids 912-1750	1,7,9-17
X	EMBL/GENBANK DATABASES Accession no C06247 Sequence reference HSC2476 25 August 1996 TAKEDA J: "EST" XP002122459 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA707194 Sequence reference AA707194 5 January 1998 HILLIER L ET AL:"WashU-NCI human EST Project" XP002122460 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA599596 Sequence reference AA599596 29 September 1997 HILLIER L ET AL: "WashU-NCI human ESR project" XP002122461 the whole document	3-5
X	KATSURA K ET AL: "IDENTIFICATION OF THE PROTEOLYTIC ENZYME WHICH CLEAVES HUMAN P75 TNF RECEPTOR IN VITRO" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 222, no. 738, 15 May 1996 (1996-05-15), pages 298-302, XP002058218 ISSN: 0006-291X page 299, paragraphs 2,3	25
A	EP 0 657 536 A (YEDA RES & DEV) 14 June 1995 (1995-06-14) cited in the application	

-/—

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PORTEU F ET AL: "HUMAN NEUTROPHIL ELASTASE RELEASES A LIGAND-BINDING FRAGMENT FROM THE 75-KDA TUMOR NECROSIS FACTOR (TNF) RECEPTOR" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 28, 5 October 1991 (1991-10-05), pages 18846-18853, XP000229747 ISSN: 0021-9258	
P,X	WO 98 20140 A (GRANGER GALE A ;UNIV CALIFORNIA (US); GATANAGA TETSUYA (US)) 14 May 1998 (1998-05-14) cited in the application the whole document	1,7, 9-17, 19-23, 25-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 10793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 21-23 and 30-32 are (partially) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 99/10793

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0657536 A	14-06-1995	AU 679559 B	03-07-1997
		AU 7574294 A	04-05-1995
		CA 2133872 A	13-04-1995
		JP 7194376 A	01-08-1995
		US 5665859 A	09-09-1997
		US 5766917 A	16-06-1998
		ZA 9407962 A	21-11-1995
WO 9820140 A	14-05-1998	AU 5162198 A	29-05-1998
		EP 0938548 A	01-09-1999
		NO 992187 A	01-07-1999

BC

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
220002057756		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 10793	14/05/1999	14/05/1998

Applicant

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 10793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 21-23 and 30-32 are (partially) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/10793

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N15/12 C07K14/47 C12N9/64 C12N15/11
C07K16/18 C07K16/40 C12Q1/68 G01N33/68 G01N33/573
C12Q1/37 A61K38/17 A61K38/48 A61K48/00 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no AJ003355 Sequence reference HSJ003355 13 October 1997 SZULZEWSKY I ET AL: "An integrated transcript map for the whole human chromosome 21" XP002121991 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA779203 Sequence reference AA779203 6 February 1998 HILLIER L ETAL: "WashU-NCI human EST project" XP002122454 the whole document	3-5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 1999	03/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van der Schaal, C

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no U52222 Sequence reference HS5252210 26 April 1996 KANOH H AND EXTON J: "Human afarpatin 2, a putative target protein of ADP-ribosilation" XP002122455 the whole document	1,7,9-17
X	MINET M AND LACROUTE F: "Cloning and sequencing of a human cDNA coding for a multifunctional polypeptide of the purine pathway by complememntation of the ade2-101 mutant in Saccharomyces cerevisiae" CURRENT GENETICS, vol. 18, 1990, pages 287-291, XP002122452 figure 3	1,7,9-17
X	GONZALEZ I ET AL: "Variation among human 28S ribosomal genes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 82, November 1985 (1985-11), pages 7666-7670, XP002122453 WASHINGTON US figure 3	1
X	EMBL/GENBANK DATABASES Accession no T33896 Sequence reference HS89620 14 January 1995 ADAMS M ET AL: "Initial assessment of human gene diversity and expression patters based upon 52 ...." XP002122456 the whole document	3-5
P,X	EMBL/GENBANK DATABASES Accession no AI002979 Sequence reference AI002979 11 June 1998 HILLIER L ET AL: "WashU-NCI human EST project" XP002122457 figure W	3-5
X	EMBL/GENBANK DATABASES Accession no AA806165 Sequence reference AA806165 16 February 1998 "National Cancer Insitiute, Cancer Genome Anatomy Project" XP002122458 the whole document	3-5

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3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OSHIMA A ET AL: "The human cation-independent mannose 6-phosphate receptor" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 263, no. 5, 15 February 1988 (1988-02-15), pages 2553-2562, XP002121990 MD US see figure 2 amino acids 912-1750	1,7,9-17
X	EMBL/GENBANK DATABASES Accession no C06247 Sequence reference HSC2476 25 August 1996 TAKEDA J: "EST" XP002122459 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA707194 Sequence reference AA707194 5 January 1998 HILLIER L ET AL:"WashU-NCI human EST Project" XP002122460 the whole document	3-5
X	EMBL/GENBANK DATABASES Accession no AA599596 Sequence reference AA599596 29 September 1997 HILLIER L ET AL: "WashU-NCI human ESR project" XP002122461 the whole document	3-5
X	KATSURA K ET AL: "IDENTIFICATION OF THE PROTEOLYTIC ENZYME WHICH CLEAVES HUMAN P75 TNF RECEPTOR IN VITRO" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 222, no. 738, 15 May 1996 (1996-05-15), pages 298-302, XP002058218 ISSN: 0006-291X page 299, paragraphs 2,3	25
A	EP 0 657 536 A (YEDA RES & DEV) 14 June 1995 (1995-06-14) cited in the application	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PORTEU F ET AL: "HUMAN NEUTROPHIL ELASTASE RELEASES A LIGAND-BINDING FRAGMENT FROM THE 75-KDA TUMOR NECROSIS FACTOR (TNF) RECEPTOR" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 28, 5 October 1991 (1991-10-05), pages 18846-18853, XP000229747 ISSN: 0021-9258	
P,X	WO 98 20140 A (GRANGER GALE A ;UNIV CALIFORNIA (US); GATANAGA TETSUYA (US)) 14 May 1998 (1998-05-14) cited in the application the whole document	1,7, 9-17, 19-23, 25-32

INTERNATIONAL SEARCH REPORT

[redacted]nation on patent family members

[redacted]rnational Application No
PCT/US 99/10793

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0657536 A	14-06-1995	AU 679559 B	03-07-1997
		AU 7574294 A	04-05-1995
		CA 2133872 A	13-04-1995
		JP 7194376 A	01-08-1995
		US 5665859 A	09-09-1997
		US 5766917 A	16-06-1998
		ZA 9407962 A	21-11-1995
WO 9820140 A	14-05-1998	AU 5162198 A	29-05-1998
		EP 0938548 A	01-09-1999
		NO 992187 A	01-07-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
N.78491 DMG/PJC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/10793	14/05/1999	14/05/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/57		
Applicant		
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/12/1999	15.09.00
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Buchet, A Telephone No. +49 89 2399 7401

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/10793

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-47 as originally filed

Claims, No.:

1-36 with telefax of 18/08/2000

Drawings, sheets:

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 21-23 (partially), 30-32.

because:

☒ the said international application, or the said claims Nos. 21-23 (partially), 30-32 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6, 8, 12, 16-35
	No:	Claims	1-5, 7, 9-11, 13-15, 36
Inventive step (IS)	Yes:	Claims	8
	No:	Claims	1-7, 9-36
Industrial applicability (IA)	Yes:	Claims	1-20, 21-23 partially, 24-29, 33-36
	No:	Claims	21-23 partially, 30-32

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: WO 98/20140
D2: Biochemical and Biophysical Research Communications
vol. 222, n° 738, 1996, pp 298-302
D3: EP 0 657 536 A
D4: EMBL/GENBANK Databases
Accession n° AA779203, Sequence reference AA779203
D5: Current Genetics
vol. 18, 1990, pp 287-291
D6: Proceedings of the National Academy of Sciences of USA
vol. 82, 1985, pp 7666-7670
D7: EMBL/GENBANK Databases
Accession n° T33896, Sequence reference HS89620
D8: EMBL/GENBANK Databases
Accession n° AA806165, Sequence reference AA806165
D9: Journal of Biological Biochemistry
vol. 263, n°5, 1988, pp 2553-2562
D10: EMBL/GENBANK Databases
Accession n° C06247, Sequence reference HSC2476
D11: EMBL/GENBANK Databases
Accession n° AA707194, Sequence reference AA707194
D12: EMBL/GENBANK Databases
Accession n° AA599596, Sequence reference AA599596

Re Item I
Basis of the report

This report is also established on the basis of pages 1-46 of the sequence listing (SEQ ID NOS: 1-154).

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and

industrial applicability

For the assessment of the present claims 21-23 and 30-32 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 21-23 (partially) and 30-32 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item IV
Lack of unity of invention

Taking into account the argument brought in Item VIII-1, it is considered that the only common concept linking together the polynucleotides and polypeptides generally claimed in this application is that they lead to increased cleaving and releasing of TNF-receptors. This encompasses a large number of molecules for which some examples are given in claims 2 and 8. This concept is however not inventive, see e.g. D3 and Item V-2 below. It is considered that the different specified molecules represent different inventions. Therefore, the present application lacks unity (Rule 13 PCT).

Re Item V
Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Novelty:

- D7 discloses a human cDNA fragment which displays 99.7% identity in 355 bp overlap

with the sequence SEQ. ID NO:5 of the present invention. Similarly, D11 discloses a human cDNA fragment which displays 99.6% identity in 492 bp overlap with the sequence SEQ. ID NO:9.
- The elucidation of the sequences of D7 and D11 implies that the corresponding DNA fragments were isolated and sequenced. Therefore, these documents are considered to anticipate the subject-matter of claims 3 to 5 so far as relating to SEQ. ID NO:5 and SEQ. ID NO:9, respectively.

- D4 discloses a cDNA fragment which displays 99.6% identity in 245 bp overlap (or 100% over 235 bp) with the coding region of SEQ. ID NO:1. D6 discloses a rRNA fragment which displays 96.7% identity in 698 bp overlap (or 100% over 440 bp) with SEQ. ID NO:2. D8 discloses a cDNA fragment which displays 99.3% identity in 419 bp overlap with the coding region of SEQ. ID NO:6. D10 discloses a cDNA fragment which displays 98.3% identity in 460 bp overlap with the coding region of SEQ. ID NO:8. D12 discloses a cDNA fragment which displays 99.8% identity in 417 bp overlap with the coding region of SEQ. ID NO:10.
- As presently formulated (see Item VIII) and assuming that the homologue region displays the claimed activity, D4, D6, D8, D10 and D12 are considered to anticipate the subject-matter of claims 1-5 and 36 so far as relating to SEQ. ID NO:1, SEQ. ID NO:2, SEQ. ID NO:6, SEQ. ID NO:8 and SEQ. ID NO:10, respectively.

- D5 discloses the DNA sequence of a 1,5 kb cDNA insert able to complement the ade2-101 mutant in *Saccharomyces cerevisiae*, as well as the deduced sequence of the 425 amino acid predicted protein (p 289, Fig. 3a).
- A sequence comparison reveals that SEQ. ID NO:150, which corresponds to the coding sequence of SEQ. ID NO:4, shares 100% identity in 258 aa overlap with the sequence disclosed in D5. The new function disclosed in claims 1 and 36 are merely considered as an inherent feature of the corresponding known sequences. Therefore, D5 is novelty-destroying for claims 1-5, 7, 9-11 and 36.

- D9 discloses the full length cDNA for the human cation-independent mannose 6-phosphate receptor and its predicted amino acid sequence (p 2555-2556, Fig. 2). Expression of the cDNA in transfected COS cells produced a cell-surface protein which was further purified by affinity column (p 2558, Fig. 5).
- A sequence comparison reveals that SEQ. ID NO:152, which corresponds to the

coding sequence of SEQ. ID NO:7, shares 99.9% identity in 839 aa overlap with the sequence disclosed in D9. The new function disclosed in claims 1 and 36 are merely considered as an inherent feature of the corresponding known sequences. Therefore, D9 is novelty-destroying for claims 1-5, 7, 9-11, 13-15 and 36.

- For the reasons mentioned above (see also Item VIII), claims 1-5, 7, 9-11, 13-15 and 36 do not fulfil the requirements of Article 33.2 PCT.

2) Inventive step:

- D2 isolates the enzymatic activity responsible for the cleavage of human p75 TNF-R, named TRRE. It was identified in the culture supernatant of PMA-stimulated THP-1 cells (p 299, table 1), which constitutes the first stable TRRE source. This activity is partially inhibited by chelating agents, as demonstrated by measurement of the TRRE activity on COS-1 cells transfected with a construct harboring the human p75 TNF-R cDNA (p 302, Table 2).

- D3 purifies the protease responsible for the shedding of the TNF-R, which activity is monitored by the TNF binding capacity of COS-7 cells transformed with TNF-R constructs (p 6). For example, the detergent extracts of membranes isolated from cells activated with PMA are applied to an affinity purification column which resin is covalently linked with a fragment of the receptor (p 8, l 46-51). Antibodies can be raised against this purified protein. Mutant cells deprived of the activity can be transformed with a genomic or cDNA library in order to clone the corresponding gene (p 9, l 45-54). The protease (claim 1), a method for its production (claim 7) and its purification (claim 3), the encoding DNA molecule (claim 4), an antibody (claim 11) or an inhibitor (claim 10) directed to this protease, derived pharmaceutical composition (claims 8 and 16-19) are claimed.

- Activities responsible for the shedding of TNF-R have already been reported in D2 and D3. Methods to isolate corresponding proteins and/or identify encoding genes are readily available and would be used by the man skilled in the art. Therefore, in the light of these documents, the general concept as disclosed e.g. in claims 1, 7 or 33-36 can not be considered as inventive per se. The fact that D2 and D3 do not fully report the TRRE purification or the isolation of the corresponding gene is not sufficient for

recognising an inventive activity for the general concept. Furthermore, it is noted that the present application does not contribute to this particular aspect neither.

- Presently, D2 is considered to deprive claims 7, 9, 11-12, 21, 23, 25 and 34 of any inventive activity. The same applies to D3 with respect to claims 1, 3, 7, 9, 11-17, 21-23, 26-28, 30 and 32-36.
- Furthermore, claims 6, 18-20, 24, 29 and 31 do not contain further inventive matter which goes beyond usual skill.

- As presently formulated (see also Item VIII), claims 1-7 and 9-36 do not fulfil the requirements of Article 33.3 PCT.

- However, an inventive activity could be acknowledged for the whole sequences specified in claim 2 and for all product and method claims (e. g. claim 8) directly relating to these sequences.

- The technical problem to be solved by the present invention, as well as by D3 considered as the closest prior art, is to provide polynucleotides and corresponding polypeptides, which expression leads to an increased cleaving and releasing of TNF receptors.

- The solutions disclosed in the present invention are the sequences identified in claim 2 and the corresponding proteins claimed in claim 8.

- Even if these sequences were already available (see D4-D12), at least partially, there was no indication that these genes could have the claimed function. Furthermore, the inventors were the first to perform such a screening and it could not be expected to find out the claimed genes.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 98/20140	14.05.98	05.11.97	06.11.96

Re Item VII

Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2, D4-D12 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1) The polynucleotides claimed in claims 1 and 32-36 are considered to be defined by unusual parameters which do not allow to differentiate them from the prior art.
A product should be defined by all its essential technical features (Article 6 PCT), i. e. in the case of polynucleotides by their sequence. This is not the case in the present claims 1 and 32-36 which are defined in terms of the result to be achieved or the desired function.

2) The parameter introduced in claim 11 appears to be an essential feature (Article 6 PCT) so that the polypeptides of claims 7-10 are solutions to the technical problem to be solved.

CLAIMS

What is claimed as the invention is:

1. An isolated polynucleotide comprising a nucleotide sequence with the following properties:
   a) the sequence is expressed at the mRNA level in Jurkat T cells;
   b) when COS-1 cells expressing TNF receptor are genetically altered to express the sequence, the cells have increased enzymatic activity for cleaving and releasing the receptor.

2. The polynucleotide of claim 1, wherein the nucleotide sequence is contained in a sequence selected from the group consisting of
   a) SEQ. ID NO:1;
   b) SEQ. ID NO:2 or SEQ. ID NO:3;
   c) SEQ. ID NO:4;
   d) SEQ. ID NO:5;
   e) SEQ. ID NO:6;
   f) SEQ. ID NO:7;
   g) SEQ. ID NO:8;
   h) SEQ. ID NO:9; and
   i) SEQ. ID NO:10.

3. An isolated polynucleotide comprising at least 30 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to any of claims 1-3

4. An isolated polynucleotide comprising a linear sequence of at least 50 consecutive nucleotides at least 90% identical to a sequence contained in said nucleotide sequence of the polynucleotide of claim 1.

5. An isolated polynucleotide of at least 50 nucleotides capable of hybridizing specifically to said nucleotide sequence of a polynucleotide according to any of claims 1-3 at 68°C in 0.5 M phosphate buffer pH 7, 7% SDS, and 100 μg/mL salmon sperm DNA, followed by washing in a buffer containing 3X SSC.

6. An antisense polynucleotide or ribozyme comprising at least 10 consecutive nucleotides in said nucleotide sequence of a polynucleotide according to claim 1 or 2, which inhibits the expression of a TRRE modulator.

7. An isolated polypeptide comprising an amino acid sequence encoded by a polynucleotide according to any of claims 1-5.

8. The polypeptide of claim 7, selected from the group consisting of SEQ. ID NOS: 147-158.

9. An isolated polypeptide, comprising at least 10 consecutive residues in said amino acid sequence of a polypeptide according to claim 7 or 8.

10. An isolated polypeptide, comprising at least 15 consecutive amino acids which are at least 80% identical to a sequence contained in said amino acid sequence of the polypeptide according to claim 7 or 8.

11. The polypeptide of claim 7-11, which when incubated with COS-1 cells expressing TNF receptor, promotes enzymatic cleavage and release of the receptor.

12. The polypeptide of claims 7-11, which either:
    a) lacks a membrane spanning sequence; or

b) is produced by a process comprising recombinant expression in a host cell followed by purification of the polypeptide from medium in which the cell is cultured.

13. A method of producing the polypeptide according to any of claims 7 to 11, comprising the steps of:
a) culturing host cells genetically altered to express the polynucleotide according to claim 3; and subsequently
b) purifying the polypeptide from the cells.

14. The method according to claim 13, comprising harvesting culture medium following step a); and purifying the polypeptide from the culture medium by a process comprising affinity chromatography.

15. An isolated polynucleotide encoding the polypeptide of claim 8 or 9.

16. An isolated antibody specific for a polypeptide according any of claims 7-11.

17. A method for producing the antibody according to claim 16, comprising immunizing a mammal or contacting an immunocompetent cell or particle with a polypeptide according to claim 9 or 10.

18. An assay method of determining altered TRRE activity in a cell or tissue sample, comprising the steps of:
a) contacting the sample with the polynucleotide of claim 4 or 5 under conditions that permit the polynucleotide to hybridize specifically with nucleic acid that encodes a modulator of TRRE activity, if present in the sample; and
b) determining polynucleotide that has hybridized as a result of step a), as a measure of altered TRRE activity in the sample.

19. An assay method for determining altered expression of a modulator of TRRE activity in a cell or tissue sample, comprising the steps of:
a) contacting the sample with the antibody of claim 16 under conditions that permit the antibody to bind the modulator if present in the sample, thereby forming an antibody-antigen complex; and
b) determining complex formed in step a), as a measure of the modulator.

20. A method for assessing a disease condition associated with altered TRRE activity in a subject, comprising determining altered TRRE activity in the sample from the subject according to claim 18, or determining altered expression of a TRRE modulator according to claim 19, and then correlating the extent of alteration with the disease condition.

21. A method for decreasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polypeptide according to any of claims 7-8 and 11-12, or with a polynucleotide according to any of claims 1-3 and 15.

22. A method for increasing signal transduction from a cytokine into a cell, comprising contacting the cell with a polynucleotide according to claim 6, or with an antibody according to claim 16.

23. The method according to claim 21 or claim 22, wherein the cytokine is TNF.

24. A method for screening polynucleotides for an ability to modulate TRRE activity, comprising the steps of:
a) providing cells that express both TRRE and the TNF-receptor;
b) genetically altering the cells with the polynucleotides to be screened;
c) cloning the cells genetically altered in step b); and

d) identifying clones that enzymatically release the receptor at an altered rate.

25. A method for screening substances for an ability to affect TRRE activity, comprising the steps of:

a) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the presence of the substance;

b) incubating cells expressing TNF receptor with a polypeptide according to claim 9 in the absence of the substance;

c) measuring any TNF receptor released from the cells in steps a) and b); and

d) correlating an increase or decrease of the receptor released in step a) relative to that in step b) with an ability of the substance to enhance or diminish TRRE activity.

26. Use of a polypeptide according to any of claims 7-8 or 11-12, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

27. Use of a polynucleotide according to any of claims 1-3, 6, or 15 in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

28. Use of an antibody according to claim 16, in the preparation of a medicament for treatment of the human or animal body by surgery or therapy.

29. Use of a polypeptide according to any of claims 7-8 and 11-12, a polynucleotide according to any of claims 1-3 and 15 or an antibody according to claim 16, in the preparation of a medicament for treatment of a

disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis.

30. A method of treating cancer in a subject, comprising increasing signal transduction from TNF into cells at the site of the cancer in the subject according to claim 22 or 23.

31. A method of treating a disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis, comprising decreasing signal transduction from TNF into cells at the site of the disease in the subject according to claim 21 or 23.

32. The method of claim 31, comprising administering to the subject an effective amount of the polypeptide of any of claims 7-8 or 11-12.

AMENDED CLAIMS

[received by the International Bureau on 2 February 2000 (02.02.00); original claims 33-35 added; remaining claims unchanged (1 page)]

disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis.

30. A method of treating cancer in a subject, comprising increasing signal transduction from TNF into cells at the site of the cancer in the subject according to claim 22 or 23.

31. A method of treating a disease selected from the group consisting of heart failure, cachexia, inflammation, endotoxic shock, arthritis, multiple sclerosis, and sepsis, comprising decreasing signal transduction from TNF into cells at the site of the disease in the subject according to claim 21 or 23.

32. The method of claim 31, comprising administering to the subject an effective amount of the polypeptide of any of claims 7-8 or 11-12.

33. The polynucleotide according to any of claims 1-5, wherein said nucleotide sequence is not contained in any of the sequences of the following GenBank Accession Nos: AJ003355, AA806165; AI002979; T33896; U52522; AA779203; C06247; AA707194; AA599596; 5453538; U13369; and J03528.

34. The polypeptide according to any of claims 7-10, the sequence of which is not completely encoded by a polynucleotide sequence contained in any of the sequences of the following GenBank Accession Nos: AJ003355, AA806165; AI002979; T33896; U52522; AA779203; C06247; AA707194; AA599596; 5453538; U13369; and J03528.

35. The polynucleotide according to claim 15, the sequence of which is not contained in any of the sequences of the following GenBank Accession Nos: AJ003355, AA806165; AI002979; T33896; U52522; AA779203; C06247; AA707194; AA599596; 5453538; U13369; and J03528.

C12N 9/64, 15/11, C07K 16/18, 16/40, C12Q 1/68, G01N 33/68, 33/573, C12Q 1/37, A61K 38/17, 38/48, 48/00, 39/395

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